Field Demonstration of NRL Environmental Flow Immunosensor for Environmental Monitoring

Center for Bio/Molecular Science and Engineering
Naval Research Laboratory

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for SERDP Project CU-28
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13. ABSTRACT ***(Maximum 200 Words)***

An NRL continuous flow immunosensor, known as the FAST 2000, has been developed that is adaptable for use in a variety of environmental monitoring platforms. During the past year, the FAST 2000 instrument was tested in initial field trials at numerous military bases identified by the EPA as priority Superfund cleanup sites. The field study objective was to measure the effectiveness and efficiency of the Flow Immunosensor in performing on-site field analysis for two selective explosives, TNT and RDX. Samples containing unknown concentrations of explosives were collected from groundwater and monitoring wells at various locations and analyzed in NRL sensor with no sample pretreatment or concentration. For data validation and in conjunction with the tests performed on-site by the Flow Immunosensor, independent laboratory analyses of lab splits from the field samples and explosives standards were performed by High Performance Liquid Chromatography using the EPA SW 846 Method 8330 for explosives. In addition to the contaminated field samples, appropriate controls, blanks and interferents were tested in the lab for certification and validation data requirements. Results were evaluated based on accuracy, precision, rate of false positives/negatives, cost, time, and waste generation.

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Table of Contents

Executive Summary

An NRL continuous flow immunosensor, known as the FAST 2000, has been developed that is adaptable for use in a variety of environmental monitoring platforms. During this past year, the FAST 2000 instrument was tested in initial field trials at numerous military bases identified by the U.S.EPA as priority Superfund cleanup sites. The field study objective was to measure the effectiveness and efficiency of the Flow Immunosensor in performing on-site field analysis for two selected explosives, TNT and RDX. Samples containing unknown concentrations of explosives were collected from groundwater and monitoring wells at various locations and analyzed in the NRL sensor with no sample pretreatment or concentration. For data validation and in conjunction with the tests performed on-site by the Flow Immunosensor, independent laboratory analyses of lab splits from the field samples and explosives standards were performed by High Performance Liquid Chromatography (HPLC) using the U.S. EPA SW846 Method 8330 for explosives. In addition to the contaminated field samples, appropriate controls, blanks and interferents were tested in the lab for certification and validation data requirements. Results were evaluated based on accuracy, precision, rate of false positives/negatives, cost, time, and waste generation.

The key test for the SERDP project was conducted during the week of September 23 - 27, 1997 at Volunteer Army Ammunition Plant, Chattanooga, Tennessee. Field samples were analyzed on-site by the portable FAST 2000 immunosensor and splits were analyzed by an independent U.S. EPA-certified laboratory using SW846 Method 8330. Field trial results at the site demonstrated that the FAST is capable of detecting TNT in environmental samples with accuracy and precision. The assay system required minimal sample volume (100 uL) and analyzed samples in less than 3 minutes. The sensor was quantitative, with a lowest detectable limit (LDL) of 20 ug/mL for TNT. Statistical analysis based on regression curves comparing the Flow Immunosensor to the U.S.EPA SW846 Method-8330 results demonstrated a high degree of correlation, with slope values around 0.98.

1. INTRODUCTION

1.1 Background Information

The major component in nearly all military munitions is TNT and/or RDX, compounds which are both a potential explosive hazard to remediation workers when present at high concentrations in soil and are toxic to humans at lower concentrations. The U.S. EPA has proposed a "lifetime health advisory" level of 2.0 ng/mL TNT as the maximum limit for drinking water. The DoD has over 50 sites listed on the U.S. EPA Superfund list that are contaminated with explosives from munitions manufacture, storage, and demilitarization that do not meet these limits. TNT and RDX are mobile in the soil and, due to this mobility, are a source of groundwater contamination both on and around military sites. Remediation of water and soil at these sites requires rapid, accurate analysis of field samples at the site and in the surrounding area. Each cleanup site will require monitoring for 10-30 years, necessitating analysis of thousands of samples. Currently, samples are collected and sent to a central laboratory for analysis by RP-HPLC according to U.S. EPA SW846 Method 8330, either by direct injection or after preconcentration using an extraction procedure. Turnaround times vary from a week to a month, with laboratory costs per test ranging from $1000 to $250 respectively. Current methods of analysis of both water and soil are insufficient for decision making on-site.

On-site detection systems would reduce costs substantially, provide real-time data, simplify site characterization, and expedite remediation. The estimated cost per test for on-site test analysis would range from $3 - $38 per test, far below the $250 - $1000 current costs. For site characterization, extra samples could be tested in areas where explosive residues were first detected so that the exact distribution of pollutants could be confirmed. For remediation, rapid on-site analysis could be used to guide earth moving procedures, indicate immediately the need for charcoal filter replacement, and monitor progress of composting or other remediation operations. Small composting tests indicate that a substantial decrease in contamination is observed in 30 days. Timely determination of those levels would reduce unnecessary composting times. Overall, on-site analysis would eliminate time delays, leading to more effective use of manpower and equipment. Though commercial immunoassay test kits have recently been introduced to field testing (D-Tech, Ohmicron), they require timed reagent addition, involve multiple steps, and are not easily adapted to online monitoring requirements. Colorimetric methods, also commercially available (Ensys), have these same limitations and require large quantities of solvents and disposable materials. The NRL environmental continuous flow immunosensor, is able to analyze on-site a sample in under 5 min at a cost of $10-15 per test.

1.2 Objectives of the Field Demonstration

The primary objective of this project was (a) to demonstrate the efficacy of the immunosensor for on-site characterization of areas contaminated with explosives in both water and soil and (b) to gain validation of the method by U.S. EPA and/or other regulatory agency.

To meet these objectives, laboratory tests and field trials were conducted in the Summer of 1997 using the NRL biosensor to perform on-site analysis. In conjunction with ESTCP support, field tests were carried out at Umatilla Army Depot (UMDA), Umatilla, Oregon, SUBASE Bangor, Washington, Naval Surface Weapons Center (NSWC) Crane, Indiana and Volunteer Army Ammunition Plant (VAAP). The pivotal test for the SERDP project was conducted during the

week of September 23 - 27, 1997 at Volunteer Army Ammunition Plant, Chattanooga, Tennessee. Splits of the field samples were analyzed on-site by the portable immunosensor U.S. EPA SW846 Method 8330. Results were evaluated based on accuracy, precision, cost, time, and waste generation. In addition to the contaminated field samples, appropriate controls, blanks and interferents were tested in the lab for certification and validation data requirements.

2. TECHNOLOGY DESCRIPTION

2.1 Technology Description

2.1.1 Continuous Flow Immunosensor

The Continuous Flow Immunosensor is based on a displacement assay that utilizes antibodies specific for the analyte of interest as a means of detection. The key elements of the sensor are: 1) antibodies specific for the analyte, 2) signal molecules which are similar to the analyte but labeled with a fluorophore (usually a Cy5 dye) so they are highly visible to a fluorescence detector, and 3) a fluorescence detector. For analysis, the antibodies which specifically recognize the contaminants are immobilized on a solid support and the fluorescently labeled signal molecule is bound to them, creating an antibody/signal molecule complex. The functionalized support is placed in the sensor and connected to a water stream. A sample is then introduced to the system through the injection port. If the sample contains the target analyte, a proportional amount of the labeled signal molecule is displaced from the antibody and detected by the fluorimeter downstream. Figure 1 shows a schematic of the immunosensor operation.

Displacement assays using the laboratory version of the Continuous Flow Immunosensor have been developed for a wide range of small molecular weight compounds, including drugs, explosives, and pesticides. Existing assays for a number of environmentally relevant explosives include TNT, RDX and DNT.

A manufacturable, field-portable version of the biosensor, the FAST 2000, has been engineered by Research International, Inc. (seen in Figure 2). The FAST 2000 is a rapid and convenient system for performing displacement assays with a resolution to 5 ppb. The optically-based signal gathering capabilities are combined with precise fluidics control in a PCMCIA-based PC application. The unit can be easily carried into the field and plugged directly into a portable PC for on-site data acquisition and analysis. Analysis time for each sample is approximately 2 minutes.

The system is controlled by an advanced Windows-based software program. The hardware is designed to use a National Instruments data acquisition card (DAQCard - 1200) for gathering data from the FAST 2000 control unit. An outboard box provides convenient storage of the various fluids required to perform the assays.

The system has been developed as a complete turnkey unit: The software provides a simple menu driven interactive user interface to lead users through the steps required to successfully determine if a trace amount of analyte is present in a given sample. The software also allows the more advanced user complete control of the operational parameters for running nonstandard procedures. The hardware provides the necessary fluid storage and flow control, including

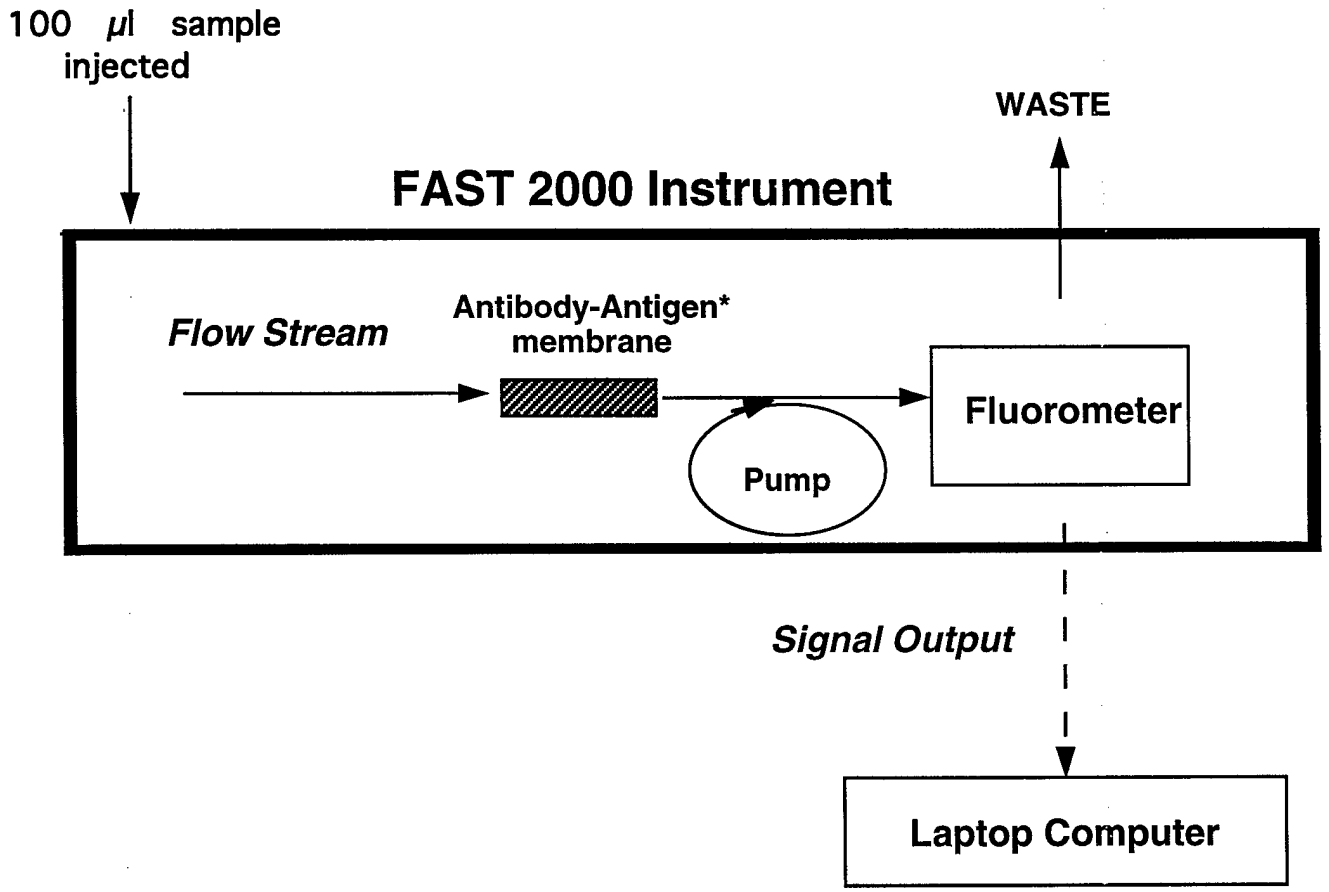
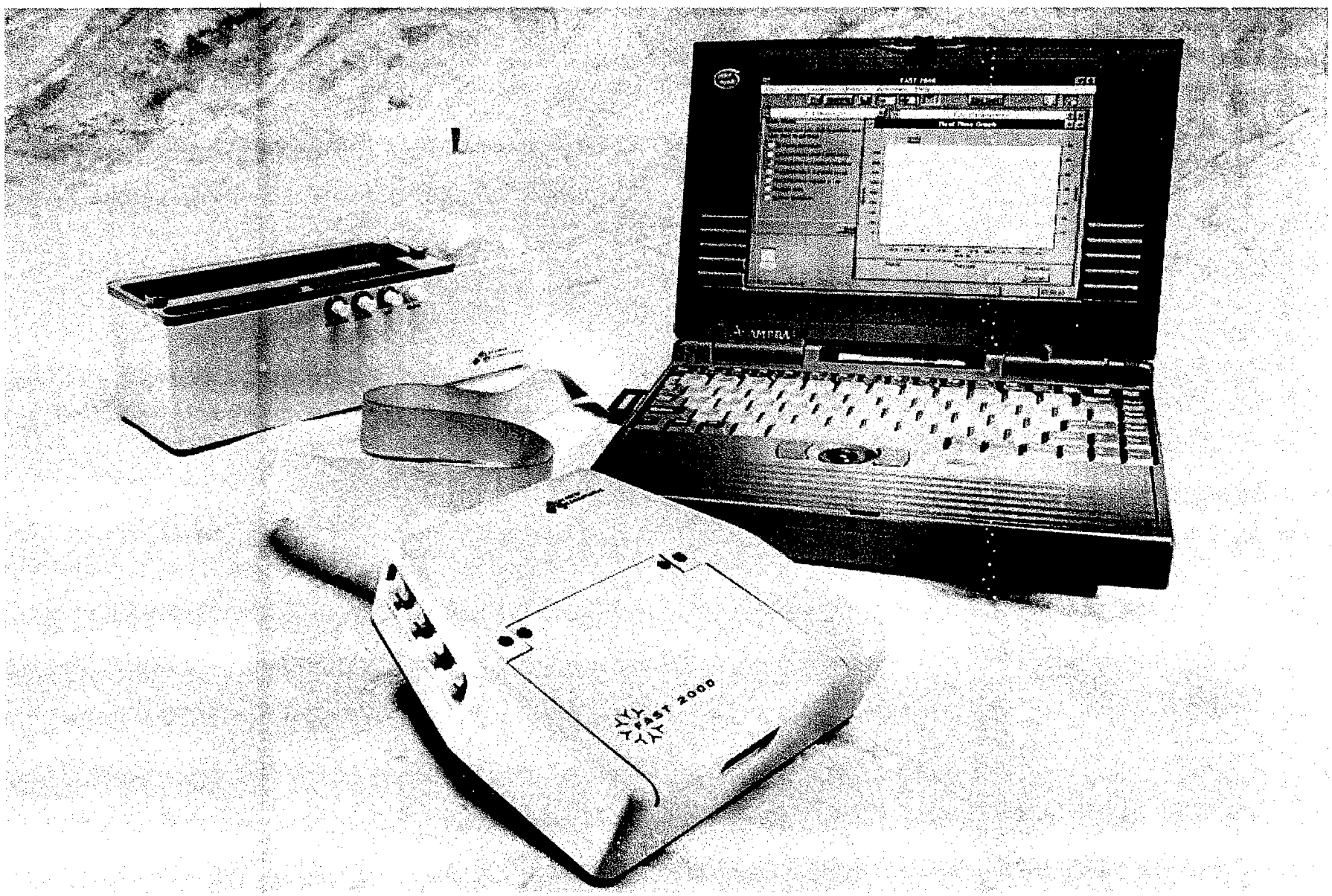


Figure 1 - Simplified schematic of the FAST 2000 Flow Immunosensor.

Figure 2



providing an automated reference standard assay that can be used anytime to calibrate the system. The reference is routinely assayed after a positive. The assays are run in disposable coupons using a robust affinity membrane to perform the displacement assay protocol.

Data analysis is made easy with the use of real time plotting of the data, data logging, and custom calibration. The Windows-based software allows for both ease of use and complex system manipulation, keeping all skill levels in mind. The assay chemistry for TNT detection has been developed to be a functional and robust system that can be successfully used in the field without the need for excessive environmental controls.

The FAST 2000 requires a computer capable of running Windows 95 or Windows 3.1 in enhanced mode. Under Windows 95, the minimum configuration is 12 MB of RAM and a 486/80 MHZ PC, while under Windows 3.1, the minimum configuration is a 486/DX 33 MHZ PC with 12 MB of RAM. In this minimum configuration, the FAST 2000 system should be the only program running. A mouse is highly recommended since not all program features are available without one. An outboard box, connected to the FAST 2000 unit via color-coded tubing, contains the waste bottle, buffer bag and reference standard bag. Before beginning as assay, flow buffer (10 mM sodium monophosphate, 2.5% ethanol and 0.01% Tween) is pumped into a buffer bag and the system is pressurized with air to control the fluid flow.

The FAST 2000 system utilizes a disposable coupon for performing the assays (seen in Figure 3). The coupon contains discrete flow channels, a membrane and filter pocket in a removable plug, pneumatically controlled valves, and septum seal area used for injecting fluids into the coupon. The coupons can be assembled with the functionalized membranes before shipping. Prior to instrument operation, the coupon is inserted into the FAST 2000 control unit, and when the handle is engaged, the coupon septum is automatically pierced. Through the Task Manager in the system software, assays are performed by a sequence of valve controls which meter the assay fluids through the coupon and into the membrane pocket. The fluids then exit the coupon and travel into the integral fluorometer in the control unit which detects any fluorescence signal present. Quantitation of the analytes, done by the system software, compares fluorescence intensity (integrated fluorescence area under the peak) to that of a reference standard.

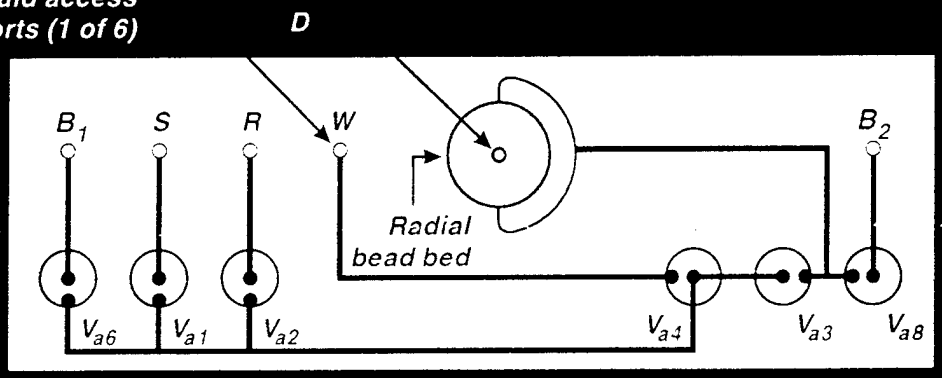
The system is equipped with the capabilities of performing a reference assay to be used as a standard for calibrating each membrane. This is referred to as the reference standard assay. The fluid is stored in a 3 mL bag in the outboard box. When 'Run a reference standard assay' is selected from the software Task Manager, the control program automatically injects fluid from the reference standard bag and performs an assay. The calculated fluorescence area under the signal peak is integrated and is used to calibrate further assay runs. Also, the reference standard assay can be used to predict the life of the membrane. Membranes that need to be replaced will have significantly reduced signal peaks.

Another method of introducing samples is to inject into the coupon through the small septum area on the top of the coupon. This reduces the sample volume required to perform an assay to 0.15 mL. Through the Task Manager, the user is instructed when to inject the sample into the coupon. Injection is done by inserting the needle of the small volume syringe through the tape cover and into the septum seal.

RESEARCH INTERNATIONAL

ASSAY COUPON HYDRAULIC ARRANGEMENT

Fluid access ports (1 of 6)

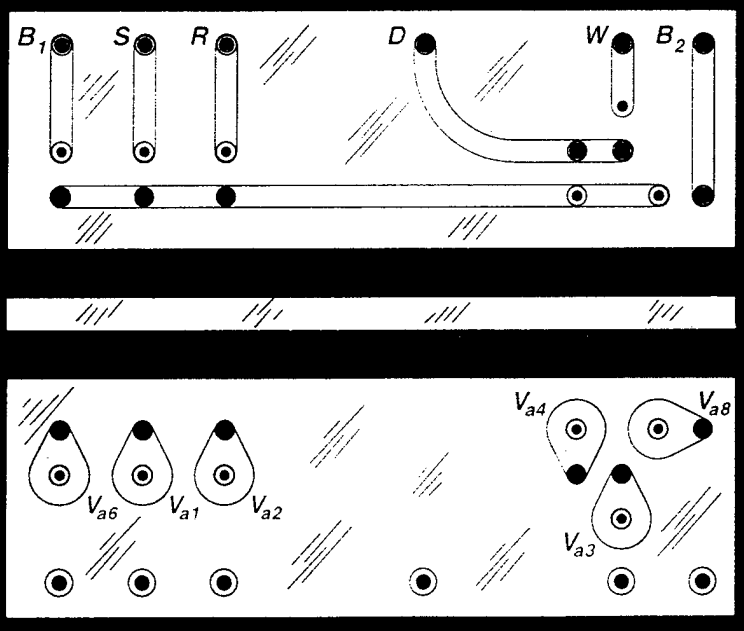


2 cm

5 - 6 cm

Nomenclature:
- B = Buffer
- R = Reagent standard
- S = Sample
- D = Detector
- W = Waste

5.81 cm



The coupon and membrane can be used for repeated assays. The life of the membrane is dependent upon the number of positive assays that were run. Since only a limited quantity of label is bound to the antibodies on the membrane, it will eventually become depleted of the label. This may take one to three days, dependent upon usage. If the standard sample cannot be detected, the membrane must be replaced.

2.2 Advantages and Limitations of the Technology

The continuous flow immunosensor is a rapid analytical tool for the detection and monitoring of compounds on-site. Little sample volume or manipulation is required for detection. The biosensor is completely portable (battery operated and lightweight), which is preferable for on-site analysis. Full set-up (from shipping box to sample analysis) takes approximately 1/2 hour.

The major strength of the NRL sensor is its adaptability for use in a variety of environments. The biosensor has been tested directly in a variety of environmental media including ground and river water, and leachate, that may or may not contain particulates with little or no affect on the overall activity of the sensor. Samples can be injected by hand in the case of groundwater or pumped from extraction wells. After soil extractions, the extracts can be added to buffer and injected.

The continuous flow immunosensor can be used either for continuous monitoring of a water stream or for testing multiple discrete samples sequentially for an extended period of time per antibody cartridge. The number of samples tested is based in part on the number of positives, since negative samples do not deplete the labeled antigen from the cartridge. For TNT and RDX, more than 50 positives can be analyzed over a single column/cartridge.

The detection limit of the instruments is already comparable to established, more complicated systems. Using the NRL sensor, TNT in water has been detected at levels of less than 5 parts-per-billion (equivalent to 5 ng/mL). This level of sensitivity is well-below that obtained using precipitation, dip stick, most enzyme immunoassays, and fluorescence polarization methods, and is comparable to radioimmunoassays.

Antibodies are recognized by biochemists and molecular biologists for their exquisite specificities. Antibody selection is based on affinity and specificity for the compound of interest. Antibodies can be selected such that the specificity is a narrow range for just one compound or wider for a group of similar compounds. Closely related compounds may also react with the antibody but usually with a lower affinity. Molecules such as TNT and RDX are too small to be antigenic so they, or a closely related analog, are coupled to a larger protein for antibody production. A larger protein cannot be coupled directly to TNT so the compound trinitrobenzene (TNB) was linked to a protein and used as the antigen to elicit antibody production. The TNT antibody used with the biosensor was produced against a TNB conjugate and selected for its affinity for TNT. Therefore, this antibody reacts with both TNT and TNB (Table 1). This poses a problem if you need to know the exact concentration of TNT in the presence of TNB. The result would be an overestimation of TNT in the sample. However, since both TNT and its degradation product TNB are toxic and explosive, this cross-reactivity is not necessarily a detriment with a screening system as both require cleanup/remediation. The RDX antibody used with the sensor, obtained from Strategic Diagnostics, Inc. (Newark, DE), was also selected for its strong affinity and low cross-reactivity with other compounds. The extent of its cross-reactivities is detailed in the company brochure but does include HMX.

One problem with any antibody-based assay is that the compound of interest must be known prior to analysis so that the appropriate antibody can be employed. Unlike HPLC which identifies a large number of compounds, an antibody recognizes only a single or limited number of compounds. Most samples contain both toxic and non-toxic components. In HPLC, both types were identified with possible swamping of the toxic compound from non-toxic compounds unless a laborious extraction procedure is followed. This problem can be eliminated using antibody-based assays because only the toxic compound generates an antibody-mediated signal.

An additional weakness of these systems is that the technologies are just now coming on the market and have not been widely tested. The Fast 2000 continuous flow sensor is being marketed by Research International and is currently undergoing field test. Background experiments and previous laboratory studies were done using a non-commercial version of the system built at NRL. The FAST 2000 flow immunosensor will be marketed by Research International for an expected price around $20K with internal components such as coupons, filters and functionalized membranes not yet in commercial production. Currently, a technically trained person is required to make the reagents and operate the system.

3. Pre-Demonstration Activities

3.1 Selecting a Site

Site selection was based on several criteria including contamination with explosives, accessability to the site, SERDP interests, and availability of non-NRL personnel for logistical support. The primary site selected for field testing was Volunteer Army Ammunition Plant (VAAP). Volunteer AAP offered support through a National Environmental Technology Test (NETTS) center which was fully equipped for field tests. The facility can provide on-site analysis using high performance liquid chromatography (HPLC) and graphite furnace atomic absorption for TNT, DNT and a range of nitroaromatic products and heavy metals. The lab also uses an automated ion analyzer for selected nitrogen and phosphorus nutrient analyses. On-site laboratory support personnel was provided by ICI Americas, Inc. (contractor operator).

3.2 Pre-Demonstration Sampling and Analysis

For preliminary measurements, the Umatilla Army Depot Activity (UMDA) site was used prior to the VAAP tests by NRL in proof-of-principle tests with the prototype biosensor. UMDA is well characterized and currently undergoing extensive remediation for groundwater contamination with TNT and RDX using pump-and-treat technology. As a result, the site provides a number of platforms for effective testing of the sensors, including a) direct measurement of contamination levels in monitoring wells, b) analysis of samples in the treatment system (pre- and post-filtration), c) testing of leachate samples, and d) direct comparisons with current field and lab measurements using the SW846 Method 8330, respectively. This initial test allowed us to recognize flaws in the original sampling scheme and provided a useful lesson in how to assure that field test results are correctly validated. To prepare for this new set of tests with the FAST 2000, NRL obtained both field blanks and unknowns from various locations to examine instrument performance and develop SOPs for field samples. Also, a trip was arranged with Dr. Judith Pennington from the Waterways

Experiment Station in Vicksburg, Mississippi, to accompany her staff on a field trial during a quarterly monitoring trip. This trip provided us with an opportunity to observe the WES sampling protocols and served as a logistics dry run for NRL personnel.

4. Site/Facility Description

4.1 Site/Facility History

The Volunteer Army Ammunition Plant (VAAP) demonstration area, located in Chattanooga, Tennessee, is a former TNT production site that covers approximately 300 acres. The area has both soil and groundwater contaminated with nitroaromatic compounds, is underlain by developed karst features and experiences short-term water table fluctuations. VAAP has 109 groundwater monitoring wells, 33 of which are in the demonstration area.

UMDA is located in eastern Oregon and is slated for closure. The base was established as an Army ordnance depot in 1941. From the 1950's until the mid- 1960's, UMDA operated an explosive washout facility to remove and recover explosives from munitions. The standard and accepted procedure at the time was to flush and drain the washout system into two unlined infiltration basins or lagoons. A 45-acre plume of RDX in the shallow groundwater aquifer near the lagoons was identified in 1981. Further investigation documented the presence of explosives in both soil and groundwater, ranging in concentration from 0-10,000 ug/L in the groundwater aquifer. These explosives included TNT, TNB, RDX and HMX. Bioremediation of the soils from the lagoons is currently underway. Treatment of the groundwater consists of pump-and-treat through granular activated charcoal filters, with re-injection of the polished water into the aquifer.

4.2 Site/Facility Characteristics

VAAP has a surface soil that is strongly acid, and categorized in the Fullerton soil group. The topsoil is 8 to 18 inches of brownish-gray chert silt loam and the subsoil is 10 to 32 inches of yellow-red silty clay loam, with slow internal drainage and low permeability. Soil thickness above the bedrock averages 30 feet.

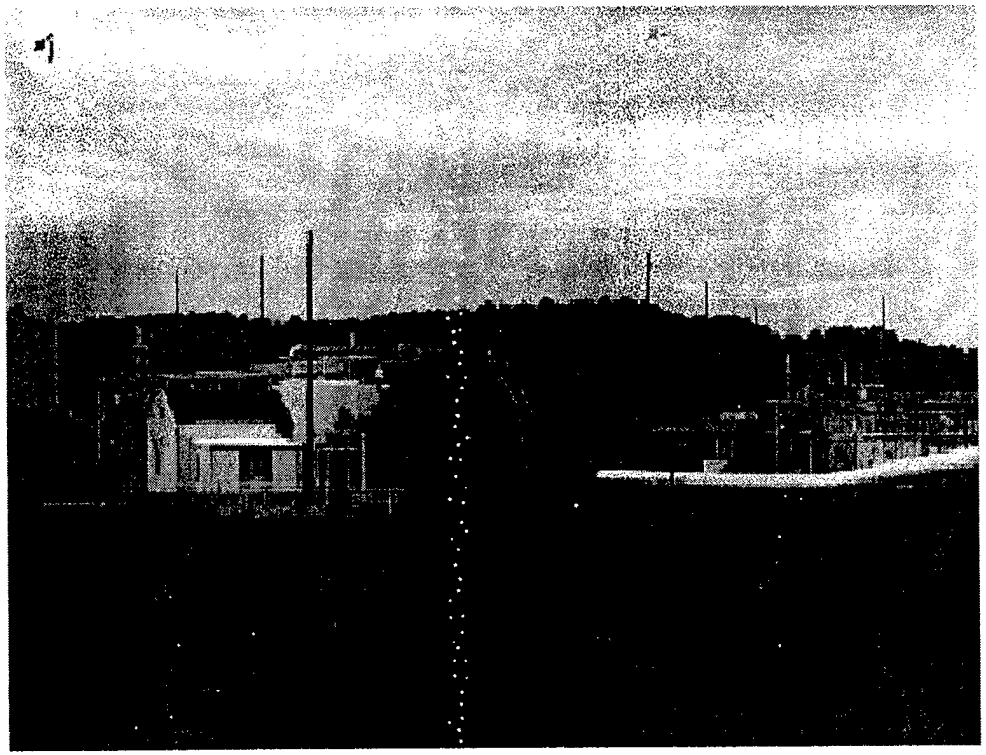
UMDA is located due east of Portland, Oregon and near the Columbia River in an arid region with no surface water. The primary geology is alluvium on top of basalt, with approximately 100 feet to groundwater. The groundwater flow is northeast to southeast, depending upon the irrigation pumping season. The net flow to southeast has led to the spread of explosives contamination. The groundwater from the contaminated region is pumped to a facility containing several GAC units. Approximately 600 gallons of water per minute is treated with the system.

5. Demonstration Approach

5.1 Performance Objectives

The objective of the field trial was the demonstration of the biosensor being operated on-site and the generation of analytical data appropriate for sensor validation and certification by a regulatory

Volunteer Army Ammunition Plant
Chatanooga, TN



TNT Manufacturing Plant

Sample Collection at Wells

agency such as the U.S. EPA or Cal EPA. A minimum of four instruments were employed for the field trial.

A specific goal for the NRL environmental immunosensor was to achieve 1-5 ppb sensitivity for TNT and RDX in environmental samples. Specificity of the sensor is provided by the antibodies immobilized on solid matrices within the biosensors. The immunosensor is specific for TNT and RDX with minimum cross-reactivities.

5.2 Demonstration Setup, Commencement, and Operation

The NRL biosensor required minimal site-preparation and instrument set-up. The sensors (minimum of four), with the computers, were transported individually in a computer carrying case. Additional supplies (sampling vials, buffers, pipets, etc) were shipped ahead in 2 small crates (approximately 18" x 30"). At the field site, a workbench approximately 2'x3' was sufficient for each sensor plus accompanying laptop computer and containers for buffer, samples and waste. A 110 V receptacle was used for instrument/computer power, though the sensors can be run for a limited time on the computer battery. Prior to the arrival of NRL personnel, samples were collected from 10-12 monitoring wells (usually by on-site technical contractors). Once collected a single person prepared the samples and injected them into the appropriate port. In general, the biosensor was operated in batch mode, with a group of samples being tested in sequence as they were collected.

5.3 Technical Performance Criteria

5.3.1 Contaminants.

Contaminants, such as other explosives which might interfere either positively or negatively were analyzed by the confirmatory laboratory. Among the contaminants expected were breakdown products of TNT and RDX primarily TNB and HMX. Other contaminants include amino-dinitrotoluenes, dinitrotoluenes, nitrotoluenes, and nitrobenzenes. We also looked for differences in solution parameters of the environmental samples compared to buffer (i.e, pH, ionic strength, color, and turbidity) to earmark those samples which posed problems with the assay so that they could be totally analyzed to aid in the identification of the interferant. Additional laboratory studies with explosives breakdown products were conducted previously to measure the extent of cross reactivity of these compounds in the TNT/RDX assays.

5.3.2 Process Waste.

Since waste from an assay may create as much of an environmental problem as the initial pollutant, the generation of waste, both liquid and solid, were monitored. Quantity as well as type of waste were monitored. During the course of 4 days of testing at VAAP, minimal waste was generated which was considered toxic or hazardous. Approximately, 1 to 2 liters of liquid were generated which contained levels of TNT above acceptable levels. Solid waste was minimal (< 20 gallon trash bag). Ways to reduce process waste to a smaller volume will be further investigated. Liquid waste contaminated with hazardous material (i.e., explosives) was handled according to OSHA guidelines. For UMDA, explosive containing liquid was filtered back through the GAC units to remove the explosives.

5.4 Factors Affecting Technology Performance.

5.4.1 Reliability.
The sensor was evaluated for factors that could effect reliability. These factors include method of shipment of instruments to the site, on-site conditions, and ruggedness of instruments, and membranes. Also, the systems were evaluated for frequency of breakdown, time required for repair, and how to determine if the systems were working properly.

5.4.2 Ease of Use.
The critical manipulations and the skill level of operator required to perform those manipulations were evaluated. The fewer the manipulations and the lower skill level of operator indicates ease of use. If necessary, sampling methods using pre-measured reagents can be prepared prior to the tests. This step would then require only that the end-user draw up a measured amount of sample from the sample vial, inject it into the sensor, and observe the results on the computer screen. Data can then be either recorded by the operator or stored in the computer for later analysis.

5.4.3 Versatility.
The ability of the biosensor to operate under a variety of environmental conditions, i.e. temperature and humidity, was evaluated to determine its versatility for various field sites. During these field trials, the biosensor only analyzed for TNT and RDX, but the sensor can be used to detect other contaminants.

5.4.4 Technology Transfer.
It is expected that the FAST 2000 continuous flow immunosensor, engineered by Research International Inc. (RI), will be commercially available within the next few months. The disposable assay cartridges are machined by RI, and the assay reagents, including the antibodies, are presently available through Strategic Diagnostics, Inc. for research purposes only. NRL, RI, and Strategic Diagnostics are currently discussing commercialization options. As a first step, NRL is currently negotiating a licensing agreement with RI to examine market potential.

5.4.5 Scaleup Issues.
The biosensor and assays were evaluated to determine what improvements are necessary for scale up operations. Can the systems be fully automated and how long can they be left unattended if fully automated? Demonstrations would provide realistic information on how long each analysis will take in the field as well as how many and how often samples are required by each field project managers to assess remediation progress. Information about supplies and support needed for this type of analysis were also gleaned from this demonstration.

5.5 Sampling Plan
The guidelines of the United States Environmental Protection Agency (US EPA) were used to comprise the following strategy and justification.

5.5.1. Selection of Analytical Laboratory.
The laboratory chosen to perform U.S. EPA SW846 Method 8330 analysis on the field samples was certified to perform these tests. Since these results are critical for analysis of the data, splits

were analyzed at the NRL and by GP Environmental Laboratory, Inc. located in Gaithersburg, Maryland (certified lab).

5.5.2. Selection of Analytical Method.

The NRL environmental immunosensors were employed for on-site analysis. Splits of the field samples were analyzed on-site by the biosensor and off-site by a certified laboratory using U.S. EPA SW-846 Method 8330. Method 8330 is a U.S. EPA-certified method for the detection of explosives in both water and soil. Also, to account for interference from nitrates, US. EPA Method 353.2 was performed on the laboratory splits.

5.5.3. Sample Collection.

At VAAP, groundwater samples from 10-15 monitoring wells in the contaminated area were collected by on-site personnel for analysis prior to biosensor testing. Samples were initially collected using a bailor into EPA-approved 1 liter amber bottles and sealed until on-site analysis or shipment to laboratories for analysis. Individual groundwater samples were collected directly from all 12 of the extraction wells. After the samples were collected, they were stored in the dark and kept cool (<10°C). Aliquots or splits from the large sample container were used for laboratory and field analyses. These aliquots (one liter for each laboratory and 40 mL for on-site analysis by the biosensor) were stored in EPA approved cleaned amber bottles in the dark and cool. Analysis for TNT was performed within one month of collection.

5.5.4. Experimental Controls.

A number of groundwater samples with minimal to no TNT content were collected to provide a representative sample of the region and serve as a control. In addition to the blank controls, spiked samples of known explosive composition were analyzed on-site by the biosensor and by the contract laboratory. To maintain sample integrity, all controls and spiked samples were stored cool (<10°C) in dark EPA-cleaned bottles or vials. To assist in the analysis of the data, many of the samples were analyzed blind. An NRL staff member assigned codes to environmental, control, and spiked samples. Certain spiked sample concentrations were known by operators to assist in the evaluation of proper system function.

5.5.5. Sample Analysis.

After collection, the field samples were analyzed on-site by NRL staff for TNT by the procedures described in Section 2.1. Splits of the field samples were shipped directly to the contract laboratory and NRL for analysis by Method 8330 as well as archival samples. Laboratory analysis via Method 8330 was performed within 1 month of sampling. A contract to a laboratory was done for each field demonstration to guard against poor performance. A minimum of seven analyses for each sample was performed with the biosensor and duplicates with Method 8330. NRL personnel ran parallel tests on separate instruments to confirm sensor results.

6. RESULTS

6.1 Laboratory Measurements

Cross-reactivity was evaluated by the immunosensor for positive and negative effects in the laboratory prior to the field demonstration. The antibody to TNT is expected to show cross-

reactivity with TNB. Other explosives and breakdown products were examined to determine the level of cross-reactivity (Table 1). For the compounds that did cross-react, concentrations required to elicit a positive response at the MDL as well as the concentration required to yield 50% inhibition compared to the standard curve are reported. The MDL calculated for the biosensor with laboratory standard solutions is 1 ug/L. MDL will vary at each field site based on sample matrix.

6.2 Summary of Field Measurements

Quantitative values obtained by the FAST 2000 and GP Environmental for the TNT contaminated groundwater samples are shown in Table 2. In general, the FAST 2000 measured values slightly lower than that of GP Environmental. This is evident in Wells 50, 66, 80 and 81. Also evident is that the TNT concentration in Well 66 was above the linear range of the immunosensor. This groundwater sample was diluted 1:10 with flow buffer to give a more accurate response by the FAST 2000. However, all data points (7 injections: 2 undiluted/ 5 diluted 1:10) were calculated to give the mean average (seen in Appendix A). Groundwater samples that were reported less than 25 ug/L were scored as below detection limit of immunosensor. Of all the groundwater samples, only Well 91 showed a much higher signal response that the value reported by GP Environmental lab. TNT concentration in Well 91 detected by the FAST 2000 was approximately 262 ug/L whereas GP Environmental reported 34.7 ug/L. Investigative work is underway to examine if this is an isolated matrix effect on the sensor, causing a high response, or a calibration error. As seen in Appendix A, factors such as mineral content and TNT breakdown products present in the groundwater could effect estimations of explosive concentrations.

6.2.1 Accuracy and Precision

To obtain certification, several criteria must be derived from the data including proportion of false positives/negatives, accuracy, precision, cross-reactivity, and performance with real environmental samples. The data from the field trials as well as laboratory results were evaluated to obtain this information.

Accuracy and precision are important factors in validating the sensor and comparing it to existing technologies. Accuracy is an indication of how closely the average value for the sensor matches the confirmatory test. To determine accuracy, the mean value of the seven replicates is divided by the value from the confirmatory test and multiplied times 100%. Precision on the other hand gives an indication on how close the replicates are to each other. To determine precision, the standard deviation of the seven replicates is divided by the mean of the replicates and multiplied by 100%.

6.2.2 Linear Regression

Performance of the biosensor for real samples compared to the U.S. EPA accepted method, SW846 Method 8330 were analyzed by two methods - linear regression and relative percent differences (RPD). Linear regression plots were constructed from plotting each biosensor method verses the Method 8330 results for each sample. A best-fit line is calculated for each system. Under ideal conditions, true accuracy has a slope = 1.0, y-intercept = zero, and a correlation coefficient (r^2) = 1.0. A slope greater than 1.0 indicates that the biosensor method generally gave higher concentrations than Method 8330, and the reverse is true for a slope less

Table I - TNT 11B3 Antibody Crossreactivity

Sample	Crossreactivity (%)
2,4,6-trinitrotoluene (TNT)	100
1,3,5-trinitrobenzene (TNB)	600
Tetryl	38
2-amino-4,6-DNT	21
2,4-dinitrotoluene (DNT)	20
Nitrobenzene (NB)	16
2-nitrotoluene (NT)	9
HMX	5
2,6-dinitrotoluene	4
4-amino-2,6-DNT	1
RDX	1

Table 2. Detection of TNT Volunteer Army Ammunition Field Trial Chattanooga, TN		
Well	FAST 2000 [TNT] ug/L	GP Environmental Lab- (8330) [TNT] ug/L
28	BDL	24.4
37	235	248
50	37	79
65	172	91.5
66	20795	30600
69	5726	3110
77	2978	7310
80	989	2610
80D	1269	2740
81	3075	990
81D	1087	NA
86	BDL	8.6
88	BDL	NA
91	262	34.7
BDL for FAST 2000 < 25 ppb		
NA = Not Analyzed		

than 1.0. The correlation coefficient indicates the amount of scatter in the data, with 1.0 being no scatter.

Exhibited in Figure 4 is a linear regression plot of TNT concentrations from groundwater samples determined by both methods (GP Environmental- HPLC and FAST 2000- fluorescence immunoassay). Results obtained from the graph show a correlation coefficient (r^2) of 0.970 which is slightly less than the ideal value of 1.0. The linear regression plot yielded a slope of 1.4, which is also slightly higher than the ideal value of 1.0. Usually a slope greater than 1.0 indicates the field method results are biased high. In general, the closer the slope is to 1.0 the better the correlation is to the best fit line. Also, the closer the y-intercept is to 0 the less bias in the data sets.

Exhibited in Figure 5 is a linear regression plot comparing SW-846 Method 8330 (HPLC) to determine TNT concentrations from Volunteer AAP groundwater samples. HPLC results by GP Environmental and NRL show some variance as indicated by a correlation coefficient (r^2) of 0.97. Figure 7 also exhibits a linear regression plot between Method 8330 performed at NRL and results obtained by the FAST 2000 while at the field site. Again, most evident is the correlation coefficient (r^2) of 0.97. This data demonstrates that the FAST 2000 and the EPA accepted HPLC method do show good agreement.

6.2.3 Relative Percent Difference (RPD)

The RPD values between Method 8330 concentrations and the field screening results were calculated with the following equation:

$$RPD = \left[\frac{(D_1 - D_2)}{(\frac{D_1 + D_2}{2})} \right] * 100$$

where D_1 = Biosensor concentration and D_2 = Method 8330 concentrations. The smaller the RPD value, the closer are the concentrations of the two methods and the more accurate the field screening method. A positive RPD indicates that the field screening method gave higher concentrations than Method 8330 results. The reverse is true for a negative RPD.

As seen in Appendix A, a mean RPD value has been calculated for the 7 injections of each groundwater sample. RPD measurements for TNT concentrations in groundwater samples were seen as low as -5.4% exhibited in Well 37. However, there were two wells with calculated RPD values of 102 and 153 (Well 81 and 91). This high RPD value could be a result of the matrix effect which may cause a high estimation of the total TNT concentration. Overall, the RPD values were reasonably close to an estimated limit of +50. However, RPD values must be in conjunction with other criteria to determine usefulness and priority. Continued testing with the biosensor is being done to improve the precision of the field samples.

Figure 5. TNT Linear Regression Plot : FAST 2000 vs QST Environmental Lab (Method 8330)

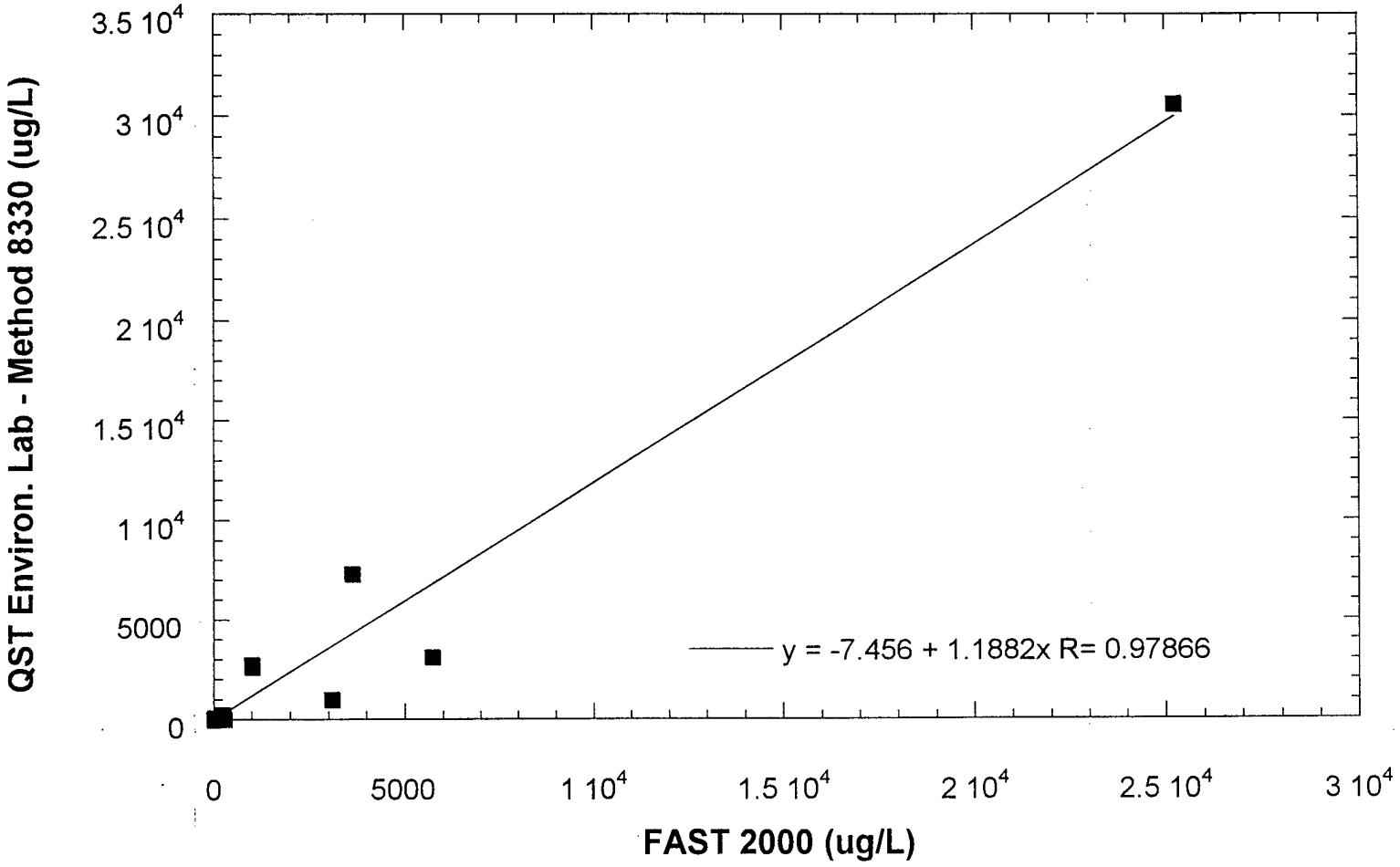


Figure 6. TNT Linear Regression Plot : NRL Method 8330 vs GP Environmental Lab Method 8330

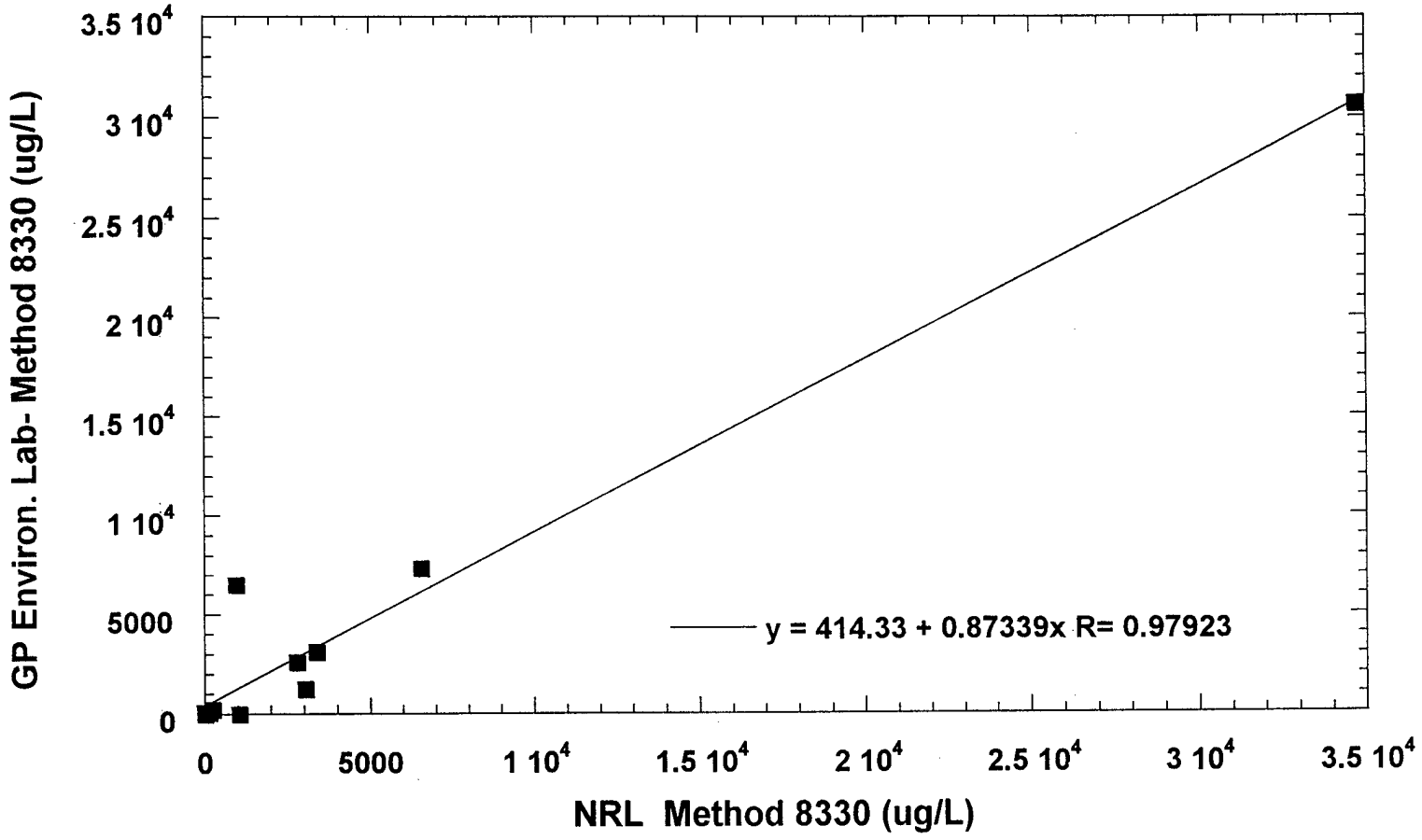
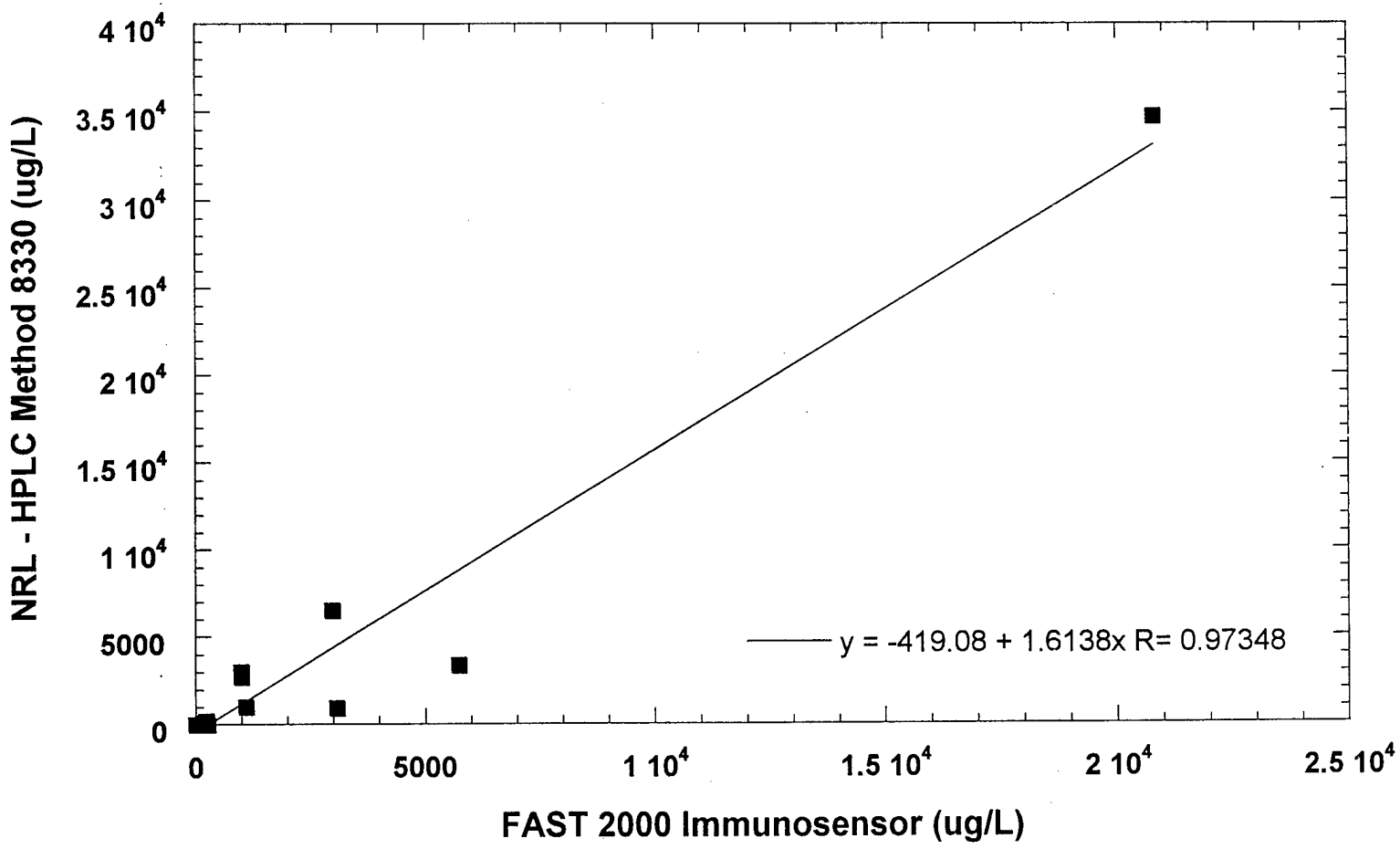


Figure 7. TNT Linear Regression Plot :
NRL HPLC Method 8330 vs Fast 2000 Immunosensor



7. Data Collection, Storage, and Archiving Procedures

7.1 Data Format

Data collected from the continuous flow immunosensor was stored on IBM formatted 3.5" high density diskettes. Diskettes can be accessed for fluorescence peak area and data integration. All groundwater sample injections and internal standard solutions were recorded on a NRL FAST 2000 data sheet that indicate date and time sample received, matrix type (groundwater or soil), sample identification, personnel who prepared sample, FAST 2000 filename, initials of FAST 2000 operator and comments related to sample injection (Appendix A). Data collected from the biosensor was recorded directly and legibly in ink on formatted data sheets. All data sheets were signed and dated in ink.

7.2 Data Storage and Archiving Procedures

Values from the data sheets were inputted into a computer spreadsheet for further analysis as well as storage. All results were stored on computer disks for archival purposes. Hardcopies of the data sheets will be maintained at NRL.

8. Cost Performance Criteria

8.1 Startup Costs

Initial costs for the NRL environmental immunosensor are now being determined. With automation and commercialization, these costs should be reduced. The initial materials and reagents needed to conduct on-site analyses are shown below:

8.1.1 Continuous Flow Immunosensor

-FAST 2000 instrument and laptop computer (one time purchase)
-assay cartridges
-activated membranes for antibody immobilization
-monoclonal antibodies specific for explosives
-fluorescent dye-labeled antigens
-other disposables (test tubes, pipets, buffer, injection syringes etc..)

8.2 Operations and Maintenance Costs

The assays were evaluated based on what supplies are routinely used to perform an analysis. In addition, the cost of the operator (time/assay times labor cost/hr) needs to be included in the operating cost and is based on the skill level required to operate the system. If routine maintenance is required, the costs involved will be evaluated. Reagent stability and shelf-life of the antibody components should be determined, as well as assay cartridge performance characteristics with repeated use.

8.3 Life Cycle Costs

U.S. EPA has estimated that a single contaminated site with current technology will take 10-30 years for groundwater remediation. It was estimated that a minimum of 200 samples will need to

be analyzed per year. To compare costs for the duration of the remediation, initial costs and cost per sample as well as maintenance costs were compared to determine the number of samples that need to be analyzed to demonstrate economic feasibility of the biosensor. The savings of time between sampling and analysis results with the biosensor also play a role in determining costs for remediation.

To date, the lifetime of the instrument and maintenance schedules have not been determined. The disposable items such as the antibody-coated membranes are factored into the cost per sample. Information was collected and evaluated during these demonstrations to aid in the determination of life-cycle costs for the biosensor.

Yearly costs with Quarterly Monitoring of 20 Wells

* 200 samples X $250/sample = $50,000 (Current technology)
* 200 samples X $15/sample = $3,000 (Biosensor)

9. Regulatory Issues

The sites selected have a well-established plan for handling samples generated on base during the test period. For those samples sent back for confirmation, the NRL intends to handle all potentially contaminated samples according to 40 CFR 261.4. The Health and Safety personnel at the NRL (Code 1200) have reviewed the proposed work and have provided the guidelines for handling, storage, and disposal of the samples, which will potentially contain some level of explosives.

10. Quality Assurance Plan

10.1 Purpose and Scope of the Plan

QA/QC measures were used to assess precision and accuracy of the analytical results and to ensure that the data meet appropriate standards for validation and certification. These measures allow the investigating team to identify, appraise and correct sources of error at any stage in the process of collection or analysis.

10.2 Data Quality Parameters

Data was assessed in terms of precision, accuracy, representativeness, comparability, and completeness (PARCC) throughout the entire project. Through the QC plan and control samples, PARCC was optimized and the errors reduced. Precision or reproducibility was measured by repeated analysis of the same sample (minimum seven times) and expressed in terms of standard deviations. Accuracy is a measurement of the degree of agreement between the measured value and the "true value". For these studies, Method 8330 results (average of a minimum of duplicates) was assumed to be the "true value" as it is accepted U.S. EPA method for explosives. Accuracy can be expressed as a percentage of the ratio of the biosensor result compared to Method 8330. Representativeness indicates the degree to which the data accurately and precisely represent the

groundwater sampled. Completeness is the quantity of valid and useful data obtained for a sample compared to the total number of data points taken for a sample. This value indicates the percentage of outliers that were not valid for data analysis. The last parameter, comparability examines the ability to compare one data set to another, especially site to site. This can be effected by different techniques, sampling crews, different sampling handling and different sampling equipment. Control samples were used to assess these parameters.

10.3 Calibration Procedures, Quality Control Checks, and Corrective Action

The following QC samples were collected for the field trials to reduce PARCC errors and provide confidence in the results of the biosensor.

10.3.1 Field Replicates

Samples were collected from one location into one container and then divided into separate containers. These were used to assess the overall heterogeneity of the sample and gave an indication of the precision of the analytical procedures.

10.3.2 Field Blanks

Laboratory-supplied water was poured into clean containers in the field at the same location where groundwater are collected. These negative controls provided information about potential sources of contamination derived from local conditions. A minimum of three field blanks were analyzed.

10.3.3 Matrix Spike Samples

Samples were spiked in the laboratory with a known concentration of the target analyte into groundwater. The personnel analyzing the sample was unaware that the sample is a laboratory spiked control. These control samples were used to verify sample matrix interferences and laboratory performance. A minimum of five samples were analyzed during this demonstration by the biosensor and splits were sent to the contract laboratory.

10.3.4 Calibration Standards

During the field trial, spiked samples of known concentration were analyzed by the biosensor to evaluate how the instrument are operating. These standards were employed to calibrate the sensors. A minimum of two calibrations standards were tested per day.

10.3.5 Duplicate Samples

These samples were taken from the same monitoring point but into different containers one right after the other. Duplicate samples were a QC check on the laboratory method for precision. A minimum of two duplicate samples were analyzed.

10.3.6 Split Laboratory Samples

This control was performed by splitting the samples for laboratory analysis and shipping each part to separate laboratories. In this demonstration, for each environmental sample one split went to the biosensor, one to the contract laboratory, one to NRL. This control was used to evaluate the contract laboratory's ability to analyze groundwater samples for explosives.

10.4 Demonstration Procedures
Standard operating procedures for the biosensor have been developed and were provided to the staff performing the analyses. These are included in the appendix.

10.5 Calculation of Quality Indicators
Data quality indicators fall into two categories, generic and assay specific. Documentation, blanks, calibration standards, comparability, confirmation analysis and replicates are all generic indicators that apply to analyses. Other indicators such as analyte specificity, non-analyte interference or cross-reactivity, environmental conditions, stability, reaction time and user friendliness are assay specific.

10.5.1 Documentation
All aspects of sample collection and analysis were documented and signed. There were chain-of-custody forms for all samples. Any deviations from the SOPs or QA/QC plan was explained and documented.

10.5.2 Blanks
Various blanks or controls were included for both biosensor and for Method 8330. These blanks included field blanks and blank groundwater samples. Results of these blank samples were used to evaluate contamination errors associated with collection, preparation and analysis.

10.5.3 Calibration Standards
Calibration standards were analyzed during the field demonstration. These standards were used to evaluate instrument operation, method sensitivity, and detection limits. The standards consisted of TNT and RDX separately and mixed.

10.5.4 Comparability
All samples were handled, prepared and analyzed by the same procedures to allow for data comparability. Any differences were documented and evaluated in final analysis.

10.5.5 Confirmation Analysis
U.S. EPA SW-846 Method 8330 was the confirmatory assay for the explosives TNT and RDX. Sample splits were shipped to a certified contract laboratory for analysis with Method 8330. Initial analysis used the direct injection method. If the sample was found to be below detection limits, preconcentration was performed. Multiple laboratories are employed to prevent lost of valid data due to errors on the part of the certified laboratory.

10.5.6 Replicates
As a precision indicator, a minimum of seven replicates per sample were analyzed on the NRL environmental immunosensor and duplicates for Method 8330. The results were employed to assess error associated with sample methodology and analytical procedures.

10.5.7 Environmental Conditions
The environmental conditions (temperature, humidity, etc) were evaluated and documented during the field trial. The effect of extreme conditions on the assay and sensor was evaluated as the conditions present themselves. In addition, both samples and standards were treated the same in regards to the environmental conditions.

10.5.8 Analyte Specificity
The specificity of the immunosensor for TNT was documented. Cross-reactivity to closely related compounds was evaluated for their impact on the assay and the results needed for decision-making. The concentration that a positive is achieve with the desired analytes as well as the cross-reactants was documented.

10.5.9 Non-analyte Interference/Crossreactivity
Interference and/or cross-reactivity from non-explosives or their breakdown products were examined. Compounds that fall into this category include nitrates, particulates, pH of sample, and temperature. Nitrate analysis was performed by the certified laboratory following U.S. EPA Method 353.2. If interference or cross-reactivity is detected, it is documented.

10.5.10 Stability
Stability of the reagents, flow cartridges both for shelf-life and length of usage were evaluated in regards to environmental conditions such as temperature, humidity and light.

10.5.11 Reaction Time
The importance of time between sample preparation and the actual analysis reading was evaluated. Since there were several instruments operating, analysis was performed at variable time points after sample preparation. If time is critical, the effect and resulting error was documented and clearly marked in the standard operating procedures.

10.5.12 User Friendliness
For ultimate employment in the field, ease of use of the sensors is important. Factors that influence user friendliness include many different reagents, dilutions, extensive sample preparation, and measurement of reagents. To minimize operator error and promote user friendliness, the minimum number of steps and reagent measurements was employed. The skill level of the operator and the amount of pre-training was examined to promote ease of use.

10.6 Performance and System Audits
Since the confirmatory tests are critical for validation of the biosensors, Method 8330 results from contract lab was evaluated by NRL to ascertain that holding times were met and quality of analysis. Contracts for 8330 analysis were let for each field demonstration to allow changes in the contract laboratory if problems occurred. In addition, all splits of samples sent to the contract laboratory were analyzed by NRL for verification.

A review of the facilities where the analysis was performed, staff training, instrument operation, sample collection, execution of assay procedures, and appropriate documentation was conducted.

11. Health and Safety Plan

In general, NRL followed the health and safety plan already in place for each installation. NRL Instruction 5100.13 C and NRL Instruction 4110.1A was followed in dealing with any explosives or hazardous materials, respectively. Overall, operational requirements for the instrument

involved non-hazardous materials and procedures. Physical requirements were minimal, since the sensor weigh only a few pounds and samples were provided directly to each worker.

12. Summary of Results and Conclusions

The recent field demonstration of the NRL Environmental biosensor (FAST 2000) has successfully proven that detection and quantitation of 2,4,6- trinitrotoluene (TNT) is possible. Groundwater samples collected from Volunteer Army Ammunition Plant (Chattanooga, TN)were analyzed on-site with the FAST 2000 immunosensor and compared to EPA SW-846 Method 8330 (HPLC) to determine the accuracy and precision of the immunosensor.

The FAST 2000 has many advantages that lends itself to on-site field environmental testing. It is portable, requires minimal space for setup and analysis, can be battery operated if in remote regions, and generates minimal hazardous waste. The immunosensor is able to examine microliter volumes of the sample, analyze the sample within a few minutes and provide accurate and quantitative data. The NRL immunosensor is a cost effective method with ability to screen a number of samples in a short period of time.

Field trial results from groundwater samples containing TNT were measured by the FAST 2000 immunosensor and compared to SW-846 Method 8330. These results exhibited good correlation between both methods with a correlation coefficient (r2) of 0.97. Relative standard deviations were also seen as low as 10.5% for some groundwater samples where a minimum of 7 injection were analyzed. Relative percent differences (RPD) were also calculated from the data and yielded results comparable to other immunoassay methods.

The FAST 2000 immunosensor has a minimum detection limit of 1.0 ug/L with standard laboratory solutions of TNT. Field samples analyzed have shown detection limits slightly higher. This is possibly a result of matrix effects (pH, salinity etc.) which may cause an increase in background noise. Methods are currently being investigated to decrease or eliminate this effect and increase the sensitivity level of the immunosensor to the part-per-trillion range.

The NRL environmental immunosensor has demonstrated that it can effectively measure groundwater samples with a good degree of accuracy with minimal reagent cost. It is portable, user-friendly and requires minimal technical training for analysis of data. The immunosensor has shown from this field demonstration that it could be an asset in remediation of contaminated sites with on-line monitoring and by providing timely quantitative results. This immunosensor is now being investigated for potential use in soil and air particulate examination.

13. References

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Appendix A

Data from Volunteer Army Ammunition Plant
Chattanooga, Tennessee

Detection of TNT (FAST 2000 Immunosensor)

Volunteer Army Amunition Site

Chattanooga, TN

Date: 9/23 - 9/27/97

Sample	Injection #	Peak Start	Peak End	Integral	TNT Conc. (ug/L)	Mean (ug/L)	Rel. Std.Dev. (ug/L)	RSD (%)	Q-Value	Reject (Y or N)	GP 8330 Conc. (ug/L)	FAST 2000 Conc. (ug/L)	RPD (%)
Well 28													
TNT 100	_1.dat	41	147	11450									
Well 28	_1.dat	ND		ND	BDL	BDL	NA	NA	NA	NA	24.4	BDL	NA
	_2.dat	ND		ND									
	_3.dat	ND		ND									
TNT 100	_2.dat	51	159	3067									
	_4.dat	ND		ND									
	_5.dat	ND		ND									
TNT 100	_3.dat	43	170	4876									
	_6.dat	ND		ND									
	_7.dat	ND		ND									
TNT 100	_4.dat	31	142	3048									
Well 37													
TNT 100	_1.dat	58	190	2759									
Well 37	_1.dat	62	254	11541	418	235	96.2	41	0.43	N	248	235	(-) 5.4
	_2.dat	67	236	8260	299								
	_3.dat	65	227	4602	167								
	_4.dat	65	204	4003	145								
	_5.dat	68	217	6503	236								
	_6.dat	68	236	5851	212								
	_7.dat	68	203	4591	166								
TNT 100	_3.dat	62	208	2157									
Well 50													
TNT 100_1	_1.dat	40	98	2511									
Well 50	_1.dat	41	59	295	12	37	20.9	56.3	0.06	N	79	37.1	(-) 72
	_2.dat	33	61	378	15								
	_3.dat	33	73	480	19								
TNT 100	_2.dat	37	99	1070									
Well 50	_4.dat	34	92	614	57								
	_5.dat	34	77	500	47								
TNT 100	_3.dat	35	83	692									
Well 50	_6.dat	36	84	418	60								
	_7.dat	27	72	344	50								
TNT 100	_4.dat	36	101	735									

Sample	Injection #	Peak Start	Peak End	Integral	TNT Conc. (ug/L)	Mean (ug/L)	Rel. Std.Dev. (ug/L)	RSD (%)	Q-Value	Reject (Y or N)	GP 8330 Conc. (ug/L)	FAST 2000 Conc. (ug/L)	RPD (%)
Well 65													
TNT 100_1	_1.dat	44	121	4405									
Well 65	_1.dat	40	139	7165	163	172	52.8	30.6	0.32	N	91.5	172	61
	_2.dat	38	147	6094	138								
	_3.dat	40	145	4952	112								
TNT 100	_2.dat	47	145	1895									
Well 65	_4.dat	40	142	3187	168								
	_5.dat	40	155	2643	139								
TNT 100	_3.dat	45	146	1055									
Well 65	_6.dat	37	164	2809	266								
	_7.dat	35	181	2280	216								
TNT 100	_4.dat	45	134	919									
Well 66			153										
TNT 1000	_1.dat	37	201	2625									
Well 66	_1.dat	42	226	23905	9106	20795	12803	61	0.078	N	30600	20795	(-) 38
	_2.dat	40	176	17516	6595								
	_3.dat (1:10)	34	180	4686	17851								
TNT 1000	_2.dat	38	172	2072									
Well 66	_4.dat (1:10)	41	181	4257	20545								
	_5.dat (1:10)	46	124	3110	15009								
TNT 1000	_3.dat	46	200	1111									
Well 66	_6.dat (1:10)	43	206	4276	38487								
	_7.dat (1:10)	41	156	4210	37893								
TNT 1000	_4.dat	49		1578									
Well 69													
TNT 1000	_1.dat	32	98	738									
Well 69	_1.dat	41	209	4040	5474	5726	1447	25	0.031	N	3110	5726	59
	_2.dat	43	193	4523	6128								
	_3.dat	43	215	4482	6073								
TNT 1000	_2.dat	35	139	1352									
Well 69	_4.dat	67	226	5105	3776								
	_5.dat	45	214	5332	3944								
	_6.dat	43	258	6600	7399								
	_7.dat	40	246	6499	7286								
TNT 1000	_3.dat	35	114	892									

Sample	Injection #	Peak Start	Peak End	Integral	TNT Conc. (ug/L)	Mean (ug/L)	Rel. Std.Dev. (ug/L)	RSD (%)	Q-Value	Reject (Y or N)	GP 8330 Conc. (ug/L)	FAST 2000 Conc. (ug/L)	RPD (%)
Well 77													
Well 77	_1.dat	38	234	108218	1222	2978	315	10.5	0.62	Y	7310	2978	84
	_1a.dat (1:10)	41	264	25575	2889								
	_2.dat (1:10)	39	265	28566	3227								
	_2a.dat (1:10)	36	265	26325	2974								
	_3.dat (1:10)	36	294	24721	2793								
	3a.dat(1:10)	36	289	25173	2844								
TNT 100	_2.dat	42	217	8852									
Well 77	_4.dat (1:10)	37	298	24962	2820								
	_4a.dat (1:10)	35	286	25786	2913								
TNT 100	_3.dat	23	263	5536									
	_5a.dat (1:10)	33	280	20694	3738								
	_6a.dat (1:10)	38	281	16671	3011								
	_7a.dat (1:10)	37	268	14214	2568								
Well 80													
TNT 100	_1.dat	48	164	4645									
Well 80	_1.dat	37	156	9229	199								
	_2.dat	36	128	4683	101								
	_3.dat	37	163	4298	93								
TNT 1000	_2.dat	35	118	2065									
Well 80	_4.dat	38	175	4735	2293	989	971	98	0.3	N	2610	989	(-) 37
	_5.dat	37	155	3332	1614								
TNT 1000	_3.dat	64	145	1761									
Well 80	_6.dat	ND		ND	ND								
	_7.dat	35	168	2669	1635								
TNT 1000	_4.dat	34	144	1632									
Well 80D													
TNT 100	_1.dat	37	94	1841									
Well 80	_1.dat	38	186	27479	1493								
	_2.dat	39	178	22640	1229								
	_3.dat	38	209	20595	1119								
TNT 100	_2.dat	51	128	2327									
Well 80	_4.dat	40	174	18101	778								
	_5.dat	38	221	20987	902								
TNT 100	_3.dat	74	183	4210									
Well 80	_6.dat	55	180	25188	598	1269	644	50	0.06	N	2740	1269	73
	_7.dat	67	237	30330	720								
TNT1000	_1.dat	38	115	2837									
Well 80	_8.dat	38	143	6902	2432								
	_9.dat	35	134	6092	2147								

Sample	Injection #	Peak Start	Peak End	Integral	TNT Conc. (ug/L)	Mean (ug/L)	Rel. Std.Dev. (ug/L)	RSD (%)	Q-Value	Reject (Y or N)	GP 8330 Conc. (ug/L)	FAST 2000 Conc. (ug/L)	RPD (%)
Well 81													
TNT 1000	_1.dat	35	105	2993									
Well 81	_1b.dat	35	133	9721	3248								
	_2b.dat	37	131	8538	2853								
	_3b.dat	39	129	6678	2231								
TNT 1000	_2.dat	42	137	2829									
Well 81	_4b.dat	34	170	9039	3195								
	_5b.dat	42	154	6297	2226								
TNT 1000	_3.dat	40	108	1553									
Well 81	_6b.dat	31	175	7502	4831	3075	878	28	0.6	N	990	3075	102
	_7b.dat	43	169	4562	2938								
TNT 1000	_4.dat	34	135	4141									
Well 81D													
TNT 1000	_1.dat	37	164	6421									
Well 81D	_1.dat	40	159	7901	1230								
	_2.dat	35	169	6650	1036								
	_3.dat	36	177	6309	983								
TNT 1000	_2.dat	38	121	3002									
Well 81D	_4.dat	37	176	5432	861								
	_5.dat	37	162	4245	781								
TNT 1000	_3.dat	36	110	2977									
Well 81D	_6.dat	38	172	4521	1519	1087	251	23	0.39	N	NA	1087	NA
	_7.dat	39	177	3572	1200								
TNT 1000	_4.dat	37	103	2431									
Well 86													
TNT 1000	_1.dat	31	112	7882									
Well 86	_1.dat	BDL		BDL	BDL	BDL	NA	NA	NA	NA	8.6	BDL	NA
	_2.dat	BDL		BDL									
	_3.dat	BDL		BDL									
TNT 1000	_2.dat	39	86	935									
Well 86	_4.dat	BDL		BDL									
	_5.dat	BDL		BDL									
TNT 1000	_3.dat	36	148	3779									
Well 86	_6.dat	BDL		BDL									
	_7.dat	BDL		BDL									
TNT 1000	_4.dat	36	157	3040									

Sample	Injection #	Peak Start	Peak End	Integral	TNT Conc. (ug/L)	Mean (ug/L)	Rel. Std.Dev. (ug/L)	RSD (%)	Q-Value	Reject (Y or N)	GP 8330 Conc. (ug/L)	FAST 2000 Conc. (ug/L)	RPD (%)
Well 88													
TNT 1000	_1.dat	31	112	7882									
Well 88	_1.dat	BDL		BDL	BDL	BDL	BDL	NA	NA	NA	NA	BDL	NA
	_2.dat	BDL		BDL									
	_3.dat	BDL		BDL									
TNT 100	_2.dat	39	86	935									
Well 88	_4.dat	BDL		BDL									
	_5.dat	BDL		BDL									
TNT 100	_3.dat	36	148	3779									
Well 88	_6.dat	BDL		BDL									
	_7.dat	BDL		BDL									
TNT 100	_4.dat	36	157	3040									
Well 91													
TNT 100	_1.dat	63	184	2021									
Well 91	_1.dat	72	238	5480	271								
	_2.dat	73	293	6704	331								
	_3.dat	73	261	4825	239								
TNT 100	_2.dat	68	214	1960									
Well 91	_4.dat	67	298	6630	338								
	_5.dat	72	300	5559	283								
TNT 100	_3.dat	72	234	1993									
Well 91	_6.dat	67	267	4195	210								
	_7.dat	75	247	3159	159	262	64	24	0.29	N	34.7	262	153
NA: Not Analyzed													
BDL: Below Detection Limit of 25 ug/L													

NRL FAST 2000 DATA SHEET

	Date	Time	No. of Samples	Matrix Type	Remarks / Condition of Samples
Received from:					
Received by :					

Sample Identification	Sample Preparation/Initials	Date	FAST2000 Filename / Initials	Comments / Concentration

GP Work Order # 9709168

SAMPLE ANALYSIS REPORT

Prepared For:

NAV_RES_LAB
CODE 6910
WASHINGTON, DC 20375

VOLUNTEER ARMY AMUNITION PLANT

Prepared By:

GP Environmental Services, Inc.
202 Perry Parkway
Gaithersburg, MD 20877

October 24, 1997

Marty Sadoughi, Laboratory Director

GP ENVIRONMENTAL SERVICES
ANALYTICAL RESULTS

Project: VOLUNTEER ARMY AMUNITION PLANT

NAV_RES_LAB
CODE 6910
WASHINGTON, DC 20375
Atten: MIKE GOODWIN

GP ENVIRONMENTAL SERVICES
202 Perry Parkway
Gaithersburg, MD 20877

Atten: Client Services
Phone: (301) 926-6802

Certified by: _____

SAMPLE IDENTIFICATION

GP ID	Client ID
9709168-01A	W80
9709168-02A	W80D
9709168-03A	W69
9709168-04A	W65
9709168-05A	W28
9709168-06A	W37
9709168-07A	W50
9709168-08A	W66
9709168-09A	W77
9709168-10A	W81
9709168-11A	W86
9709168-12A	W91
9709168-13A	W37D

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-01A
Client ID: W80
Collected: 09/25/97
Dilution: 10

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/07/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	237	6.5	
1,3-Dinitrobenzene	BQL	6.5	
2,4,6-Trinitrotoluene	2610	6.5	
2,4-Dinitrotoluene	34.5	6.5	
2,6-Dinitrotoluene	BQL	6.5	
2-Amino-4,6-dinitrotoluene	BQL	6.5	
2-Nitrotoluene	BQL	14.0	
3-Nitrotoluene	BQL	14.0	
4-Amino-2,6-dinitrotoluene	344	6.5	
4-Nitrotoluene	BQL	14.0	
HMX	BQL	14.0	
Nitrobenzene	BQL	6.5	
RDX	62.1	14.0	
Tetryl	BQL	14.0	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-02A
Client ID: W80D
Collected: 09/25/97
Dilution: 10

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/07/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	259	6.5	
1,3-Dinitrobenzene	BQL	6.5	
2,4,6-Trinitrotoluene	2740	6.5	
2,4-Dinitrotoluene	34.9	6.5	
2,6-Dinitrotoluene	BQL	6.5	
2-Amino-4,6-dinitrotoluene	BQL	6.5	
2-Nitrotoluene	BQL	14.0	
3-Nitrotoluene	BQL	14.0	
4-Amino-2,6-dinitrotoluene	353	6.5	
4-Nitrotoluene	BQL	14.0	
HMX	BQL	14.0	
Nitrobenzene	BQL	6.5	
RDX	74.5	14.0	
Tetryl	BQL	14.0	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-03A
Client ID: W69
Collected: 09/25/97
Dilution: 100

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/09/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	84.4	64.8	
1,3-Dinitrobenzene	BQL	64.8	
2,4,6-Trinitrotoluene	3110	64.8	
2,4-Dinitrotoluene	6080	64.8	
2,6-Dinitrotoluene	BQL	64.8	
2-Amino-4,6-dinitrotoluene	BQL	64.8	
2-Nitrotoluene	6570	140	
3-Nitrotoluene	673	140	
4-Amino-2,6-dinitrotoluene	BQL	64.8	
4-Nitrotoluene	4430	140	
HMX	BQL	140	
Nitrobenzene	BQL	64.8	
RDX	271	140	
Tetryl	BQL	140	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-04A
Client ID: W65
Collected: 09/25/97
Dilution: 10

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/07/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	22.2	6.5	
1,3-Dinitrobenzene	BQL	6.5	
2,4,6-Trinitrotoluene	91.5	6.5	
2,4-Dinitrotoluene	147	6.5	
2,6-Dinitrotoluene	BQL	6.5	
2-Amino-4,6-dinitrotoluene	BQL	6.5	
2-Nitrotoluene	15.8	14.0	
3-Nitrotoluene	BQL	14.0	
4-Amino-2,6-dinitrotoluene	76.3	6.5	
4-Nitrotoluene	BQL	14.0	
HMX	BQL	14.0	
Nitrobenzene	BQL	6.5	
RDX	BQL	14.0	
Tetryl	BQL	14.0	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-05A
Client ID: W28
Collected: 09/23/97
Dilution: 1

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/07/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	1.8	0.7	
1,3-Dinitrobenzene	BQL	0.7	
2,4,6-Trinitrotoluene	24.4	0.7	
2,4-Dinitrotoluene	109	0.7	+
2,6-Dinitrotoluene	BQL	0.7	
2-Amino-4,6-dinitrotoluene	12.5	0.7	
2-Nitrotoluene	258	1.4	+
3-Nitrotoluene	23.7	1.4	
4-Amino-2,6-dinitrotoluene	BQL	0.7	
4-Nitrotoluene	190	1.4	+
HMX	BQL	1.4	
Nitrobenzene	BQL	0.7	
RDX	BQL	1.4	
Tetryl	BQL	1.4	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-06A
Client ID: W37
Collected: 09/23/97
Dilution: 10

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/07/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	106	6.5	
1,3-Dinitrobenzene	BQL	6.5	
2,4,6-Trinitrotoluene	248	6.5	
2,4-Dinitrotoluene	31.4	6.5	
2,6-Dinitrotoluene	BQL	6.5	
2-Amino-4,6-dinitrotoluene	BQL	6.5	
2-Nitrotoluene	BQL	14.0	
3-Nitrotoluene	BQL	14.0	
4-Amino-2,6-dinitrotoluene	86.9	6.5	
4-Nitrotoluene	BQL	14.0	
HMX	BQL	14.0	
Nitrobenzene	BQL	6.5	
RDX	32.1	14.0	
Tetryl	BQL	14.0	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-07A
Client ID: W50
Collected: 09/23/97
Dilution: 10

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/07/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	BQL	6.5	
1,3-Dinitrobenzene	BQL	6.5	
2,4,6-Trinitrotoluene	79.0	6.5	
2,4-Dinitrotoluene	296	6.5	
2,6-Dinitrotoluene	BQL	6.5	
2-Amino-4,6-dinitrotoluene	BQL	6.5	
2-Nitrotoluene	60.8	14.0	
3-Nitrotoluene	BQL	14.0	
4-Amino-2,6-dinitrotoluene	42.9	6.5	
4-Nitrotoluene	43.3	14.0	
HMX	BQL	14.0	
Nitrobenzene	BQL	6.5	
RDX	BQL	14.0	
Tetryl	BQL	14.0	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-08A
Client ID: W66
Collected: 09/23/97
Dilution: 1000

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/09/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result		Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	1810		600	
1,3-Dinitrobenzene		BQL	600	
2,4,6-Trinitrotoluene	30600		600	
2,4-Dinitrotoluene	64700		600	
2,6-Dinitrotoluene		BQL	600	
2-Amino-4,6-dinitrotoluene		BQL	600	
2-Nitrotoluene	21300		1300	
3-Nitrotoluene	1840		1300	
4-Amino-2,6-dinitrotoluene		BQL	600	
4-Nitrotoluene	178000		1300	
HMX		BQL	1300	
Nitrobenzene		BQL	600	
RDX	600		1300	J
Tetryl		BQL	1300	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-09A
Client ID: W77
Collected: 09/23/97
Dilution: 1000

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/09/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result		Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	603		600	J
1,3-Dinitrobenzene		BQL	600	
2,4,6-Trinitrotoluene	7310		600	
2,4-Dinitrotoluene	47000		600	
2,6-Dinitrotoluene		BQL	600	
2-Amino-4,6-dinitrotoluene		BQL	600	
2-Nitrotoluene	103000		1300	
3-Nitrotoluene	8670		1300	
4-Amino-2,6-dinitrotoluene		BQL	600	
4-Nitrotoluene	68300		1300	
HMX		BQL	1300	
Nitrobenzene		BQL	600	
RDX		BQL	1300	
Tetryl		BQL	1300	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-10A
Client ID: W81
Collected: 09/23/97
Dilution: 1000

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/10/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result		Det.Lim.	Qualifier
1,3,5-Trinitrobenzene		BQL	600	
1,3-Dinitrobenzene		BQL	600	
2,4,6-Trinitrotoluene	990		600	
2,4-Dinitrotoluene	6520		600	
2,6-Dinitrotoluene		BQL	600	
2-Amino-4,6-dinitrotoluene		BQL	600	
2-Nitrotoluene	14300		1300	
3-Nitrotoluene	1210		1300	J
4-Amino-2,6-dinitrotoluene		BQL	600	
4-Nitrotoluene	10200		1300	
HMX		BQL	1300	
Nitrobenzene		BQL	600	
RDX		BQL	1300	
Tetryl		BQL	1300	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-11A
Client ID: W86
Collected: 09/23/97
Dilution: 1

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/07/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	BQL	0.6	
1,3-Dinitrobenzene	4.6	0.6	
2,4,6-Trinitrotoluene	8.6	0.6	
2,4-Dinitrotoluene	90.2	0.6	+
2,6-Dinitrotoluene	BQL	0.6	
2-Amino-4,6-dinitrotoluene	3.6	0.6	
2-Nitrotoluene	106	1.4	
3-Nitrotoluene	9.6	1.4	
4-Amino-2,6-dinitrotoluene	BQL	0.6	
4-Nitrotoluene	75.6	1.4	
HMX	BQL	1.4	
Nitrobenzene	BQL	0.6	
RDX	BQL	1.4	
Tetryl	BQL	1.4	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-12A
Client ID: W91
Collected: 09/23/97
Dilution: 2

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/10/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	3.9	1.3	
1,3-Dinitrobenzene	19.6	1.3	
2,4,6-Trinitrotoluene	34.7	1.3	
2,4-Dinitrotoluene	87.0	1.3	
2,6-Dinitrotoluene	BQL	1.3	
2-Amino-4,6-dinitrotoluene	15.2	1.3	
2-Nitrotoluene	48.5	2.8	
3-Nitrotoluene	3.3	2.8	
4-Amino-2,6-dinitrotoluene	BQL	1.3	
4-Nitrotoluene	32.1	2.8	
HMX	BQL	2.8	
Nitrobenzene	BQL	1.3	
RDX	BQL	2.8	
Tetryl	BQL	2.8	

GP ENVIRONMENTAL SERVICES
ORGANIC ANALYSIS RESULTS

GP ID: 9709168-13A
Client ID: W37D
Collected: 09/23/97
Dilution: 10

Matrix: WATER
Method: SW-846 8330
Units: ug/L

Analyst: YS
Analyzed: 10/07/97
Prepared: 09/30/97

LIQUID CHROMATOGRAPHY TARGET COMPOUNDS

Parameter	Result	Det.Lim.	Qualifier
1,3,5-Trinitrobenzene	106	6.5	
1,3-Dinitrobenzene	BQL	6.5	
2,4,6-Trinitrotoluene	242	6.5	
2,4-Dinitrotoluene	28.2	6.5	
2,6-Dinitrotoluene	BQL	6.5	
2-Amino-4,6-dinitrotoluene	BQL	6.5	
2-Nitrotoluene	BQL	14.0	
3-Nitrotoluene	BQL	14.0	
4-Amino-2,6-dinitrotoluene	80.6	6.5	
4-Nitrotoluene	BQL	14.0	
HMX	BQL	14.0	
Nitrobenzene	BQL	6.5	
RDX	31.6	14.0	
Tetryl	BQL	14.0	

G.P. Environmental Services

Possible notes and definitions for this report:

BQL = Below Quantitation Limit

J = Value is less than the reporting limits but greater than zero

P = Indicates that there is greater than 25% difference for detected pesticide/Aroclor results between the two GC columns

B = Indicates that the compound was found in the associated blank

E = Indicates that the concentration exceeded the calibration range of the instrument

U = Indicates that the compound was analyzed for but not detected, number indicates the detection limit

D = Indicates that the compound was found in an analysis at a secondary dilution factor

***** = Value obtained from a 1:5 dilution

+ = Value obtained from a 1:10 dilution

= Value obtained from a 1:20 dilution

= = Value obtained from a 1:25 dilution

^ = Value obtained from a 1:50 dilution

~ = Value obtained from a 1:100 dilution

! = Value obtained from a 1:250 dilution

@ = Value obtained from a 1:125 dilution (medium level)

$ = Value obtained from a 1:500 dilution

& = Value obtained from a 1:1000 dilution

N = Flashpoint not observed; heated to specified limit

R = Flammable at room temperature

TNTC = Too numerous to count

B.P. = Detection limit taken from boiling point

F.F. = Sample gave off flammable fumes

From: NRL
Anne Kusterbeck

NRL FAST 2000 DATA SHEET

	Date	Time	# of Samples	Matrix	Remarks / Condition of Samples
Received from: Richard Turtchell	9/25	14:00	4	groundwater	Volunteer Army Ammunition Plant Chatanooga, TN
Received by :					

Sample Identification	Sample Preparation/Initials	Date	FAST2000 Filename / Initials	Comments / Concentration
W80		9/25		
W80D		9/25		
W69		9/25		
W65				

RCVD C[illegible] 9/26/97 [illegible]

Att. 2.3

Sent by: Anne Kusterbeck
NRL

v. 7/97

NRL FAST 2000 DATA SHEET

Chain of Custody Sheet

	Date	Time	No. of Samples	Matrix Type	Remarks / Condition of Samples
Anne Kusterbeck Received from: Received by :	9/25			GROUNDWATER	Volunteer Army Ammunition Plant Chatanooga, TN

Sample Identification	Sample Preparation/Initials	Date	FAST2000 Filename / Initials	Comments / Concentration
W28	grd H_2O well / RT	9/23	Packed by QWX	
W37	"		"	
W50	"			
W66	"			
W77	"			
W81	"			
W86	"			
W91	"	"	"	
W37D	"	"	"	

#2 4.5

ENVIRONMENTAL SERVICES

SAMPLE RECEIPT CHECKLIST

W.O. No. 9704-169

Client Name _____

Date Received 4/26/97

Time Received 9:10 ~

Received By LOWS

Carrier Name Fed Ex

Prepared (Logged In) By _____ 4/26/97
Initials Date

Project VAP

Site _____

VOA Holding Blank I.D. No. _____

	YES	NO		YES	NO
Airbill/Manifest Present? No. 5939921160145	✓		Trip Blanks Received? No. of Sets _____		NA
Shipping Container in Good Condition?	✓		VOA Vials Have Zero Headspace?		NA
Custody Seals Present on Shipping Container? Condition: Good ✓ Broken _____	✓		Preservatives Added to Sample?		NA
Chain-of-Custody Present?	✓		pH Check Required? Performed By? _____		NA
Chain-of-Custody Agrees with Sample Labels?	✓		Ice Present in Shipping Container?	✓	(WET)
Chain-of-Custody Signed?	✓		Container# Temperature		
Packing Present in Shipping Container? Type of Packing Bubble wrap	✓		#1 2.3		
			2 4.5		
Custody Seals on Sample Bottles? Condition: Good _____ Broken _____		✓			
Total Number of Sample Bottles 12					
Total Number of Samples 12					
Samples Intact?	✓		Project Manager Contacted? Name: Ross/Amir		
Sufficient Sample Volume for Indicated Test?	✓		Date Contacted: 4/26/97		

Any NO response must be detailed in the comments section below. If items are not applicable to particular samples or contracts, they should be marked N/A.

COMMENTS: _____

Checklist Completed by: _____

Date 4/26/97

Table 4.15 RI/FS Data for Groundwater Monitoring Wells

	Well	28	37	39	40	48
Parameter	CRLs Units	Result	Result	Result	Result	Result
135TNB	.21 UGL	0.347	18.8	LT	13.6	LT
13DNB	.458 UGL	LT	LT	60	5.1	
246TNT	.426 UGL		78		420	LT
24DNT	.397 UGL	7.35			910	
26DNT	.6 UGL	LT	LT		1300	
Aluminum	112 UGL	228	268	164	1820	181
Arsenic	2.35 UGL	LT	LT	LT	LT	3.73
Barium	2.82 UGL	56.7	125	127	25.7	246
Beryllium	1.12 UGL	LT	LT	LT	LT	LT
Boron	230 UGL	LT	LT	LT	LT	LT
Cadmium	6.78 UGL	LT	LT	LT	LT	LT
Calcium	105 UGL	79500	82500	134000	250000	30900
Chromium	16.8 UGL	LT	LT	LT	LT	LT
Cobalt	25 UGL	LT	LT	LT	65.5	LT
Copper	18.8 UGL	LT	LT	LT	LT	LT
Iron	77.5 UGL	186	373	LT	437	155
Lead	4.47 UGL	LT	LT	LT	6.88	5.62
Magnesium	135 UGL	13400	13600	4470	46700	3630
Manganese	9.67 UGL	LT	19.5	116	18000	32.6
Mercury	.1 UGL	LT	LT	LT	0.334	LT
Nickel	32.1 UGL	LT	LT	LT	LT	LT
Potassium	1240 UGL	1370	1650	32700	6470	76400
Selenium	2.53 UGL	LT	LT	LT	12.4	LT
Silver	10 UGL	LT	LT	LT	LT	
Thallium	125 UGL	LT	133	LT	LT	LT
Vanadium	27.6 UGL	LT	LT	LT	LT	LT
Zinc	18 UGL	LT	58.3	LT	71.3	23
Cyanide	5 UGL	LT	LT	LT	40.3	LT
Alkalinity	5000 UGL	400000	230000	44000	69000	170000
Alkalinity-Bicarbonate	5000 UGL	400000	230000	44000	69000	LT
Chloride	278 UGL	3120	2710	3470	5610	634
Fluoride	153 UGL	542	539	727	2650	2140
Nitrate/Nitrit	10 UGL	5600	7000	24000	20000	150
Sulfate	175 UGL	73000	19000	42000	780000	14000
COD	10000 UGL					
TOC	1000 UGL					
TDS	10000 UGL					
TSS	4000 UGL					

Table 4.15 RI/FS Data for Groundwater Monitoring Wells

	Well	49	50	53	54	66
Parameter	CRLs Units	Result	Result	Result	Result	Result
135TNB	.21 UGL	LT	2.54	0.889	0.778	1300
13DNB	.458 UGL	3.02		LT	LT	70
246TNT	.426 UGL	LT	38.5		20	26000
24DNT	.397 UGL	110		20		42000
26DNT	.6 UGL		220	19.8	LT	29000
Aluminum	112 UGL	LT	174	213	170	217
Arsenic	2.35 UGL	LT	LT	LT	LT	LT
Barium	2.82 UGL	173	105	86.3	15.6	68
Beryllium	1.12 UGL	LT	LT	LT	LT	LT
Boron	230 UGL	LT	LT	LT	LT	LT
Cadmium	6.78 UGL	LT	LT	LT	LT	LT
Calcium	105 UGL	21600	23300	67900	26400	44300
Chromium	16.8 UGL	LT	LT	LT	LT	LT
Cobalt	25 UGL	LT	LT	LT	LT	47.4
Copper	18.8 UGL	LT	LT	LT	LT	LT
Iron	77.5 UGL	LT	138	LT	252	424
Lead	4.47 UGL	LT	LT	LT	LT	LT
Magnesium	135 UGL	1360	46700	199	37000	5500
Manganese	9.67 UGL	LT	LT	LT	34.4	2960
Mercury	.1 UGL	LT	LT	LT	LT	LT
Nickel	32.1 UGL	LT	LT	LT	LT	LT
Potassium	1240 UGL	35600	18900	38200	48500	10800
Selenium	2.53 UGL	LT	LT	LT	LT	LT
Silver	10 UGL		LT	LT		LT
Thallium	125 UGL	LT	LT	LT	LT	LT
Vanadium	27.6 UGL	LT	LT	LT	LT	LT
Zinc	18 UGL	LT	LT	LT	35.9	34.7
Cyanide	5 UGL	LT	LT	LT	LT	31.2
Alkalinity	5000 UGL	110000	170000	140000	200000	95000
Alkalinity-Bicarbonate	5000 UGL	LT	170000	LT	200000	95000
Chloride	278 UGL	3370	4500	1090	1530	6620
Fluoride	153 UGL	252	574	269	445	442
Nitrate/Nitrite	10 UGL	7800	16000	2800	6800	15000
Sulfate	175 UGL	72000	37000	15000	63000	17000
COD	10000 UGL			21000	LT	160000
TOC	1000 UGL			4700	1500	52000
TDS	10000 UGL			280000	300000	270000
TSS	4000 UGL			13000	5000	17000

Table 4.15 RI/FS Data for Groundwater Monitoring Wells

	Well	67	69	77	78	79
Parameter	**CRLs Units**	**Result**	**Result**	**Result**	**Result**	**Result**
135TNB	.21 UGL	36	140	49	68	46
13DNB	.458 UGL	12.8	12.4	2.16	LT	10.2
246TNT	.426 UGL	3900	4000	330	9300	680
24DNT	.397 UGL	43000	6300	3300		15000
26DNT	.6 UGL	33000	1800	LT	LT	3200
Aluminum	112 UGL	500000	28000	2070	330000	518
Arsenic	2.35 UGL	LT	LT	LT	4.5	LT
Barium	2.82 UGL	1150	45.6	184	630	385
Beryllium	1.12 UGL	16.5	2.25	LT	5.41	1.43
Boron	230 UGL	LT	LT	LT	445	LT
Cadmium	6.78 UGL	16.8	LT	LT	LT	LT
Calcium	105 UGL	730000	76900	77400	91400	75800
Chromium	16.8 UGL	96	LT	LT	353	LT
Cobalt	25 UGL	2110	445	60.2	124	95
Copper	18.8 UGL	966	LT	LT	234	LT
Iron	77.5 UGL	2460	492	1380	382000	896
Lead	4.47 UGL	6300	9.52	LT	410	7.12
Magnesium	135 UGL	79900	24900	21000	31200	27200
Manganese	9.67 UGL	50000	13000	4880	11000	4410
Mercury	.1 UGL	6.6	0.313	0.226	1.86	LT
Nickel	32.1 UGL	924	72.2	LT	165	61.2
Potassium	1240 UGL	11600	1890	3080	13000	2100
Selenium	2.53 UGL	LT	4.51	LT	LT	LT
Silver	10 UGL	13	LT		LT	LT
Thallium	125 UGL	LT	LT	LT	LT	LT
Vanadium	27.6 UGL	LT	LT	LT	700	LT
Zinc	18 UGL	3950	363	33	853	235
Cyanide	5 UGL	121	79.2	34.3	22	8.65
Alkalinity	5000 UGL	LT	LT	110000	540000	21000
Alkalinity-Bicarbonate	5000 UGL	LT	LT	110000	540000	21000
Chloride	278 UGL	10000	5970	5870	4140	2090
Fluoride	153 UGL	4200	1510	897	795	701
Nitrate/Nitrite	10 UGL	1600000	23000	15000	6800	44000
Sulfate	175 UGL	5770	340000	20000	76000	21000
COD	10000 UGL					
TOC	1000 UGL					
TDS	10000 UGL					
TSS	4000 UGL					

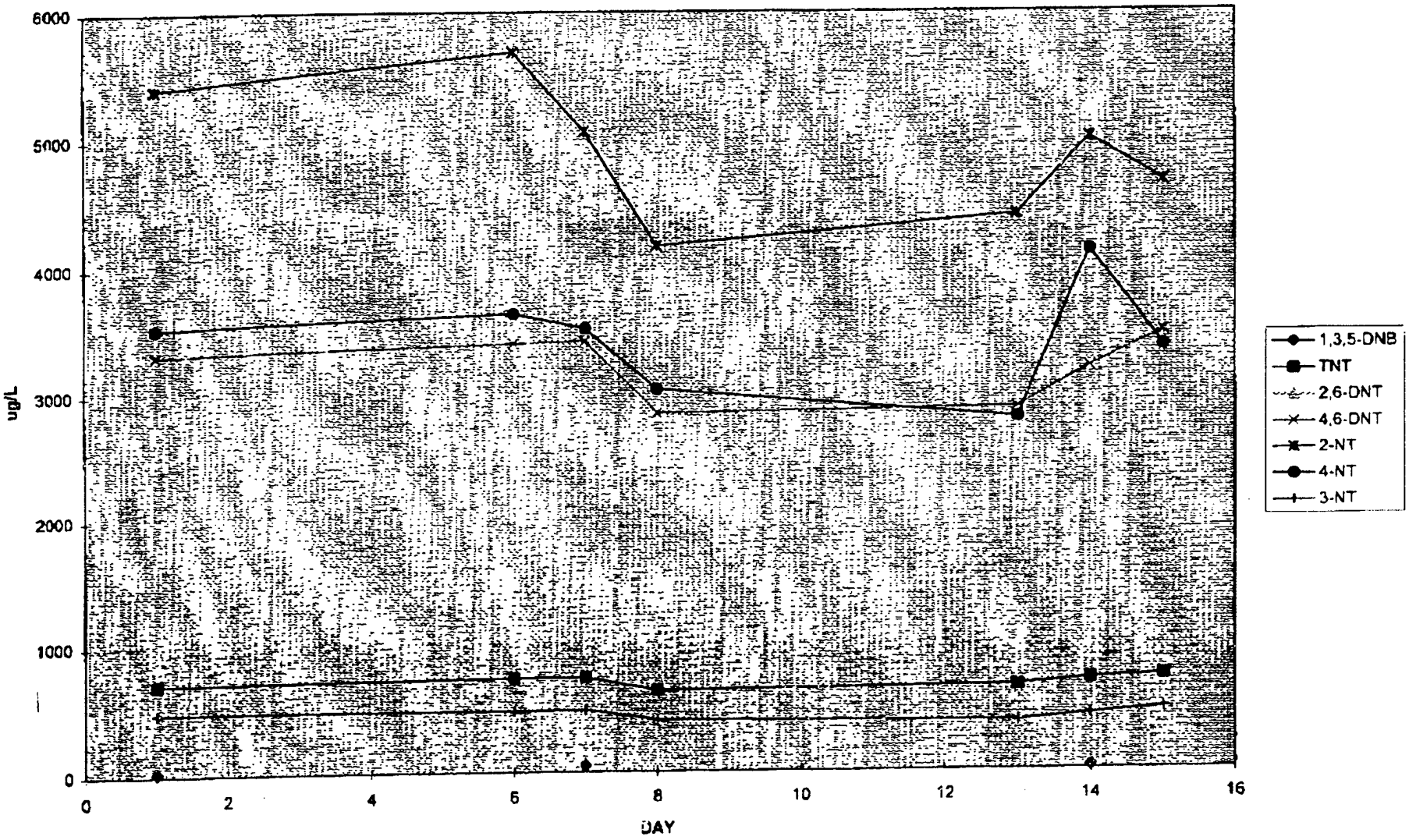
Table 4.15 RI/FS Data for Groundwater Monitoring Wells

	Well	80	81	82	83	84
Parameter	CRLs Units	Result	Result	Result	Result	Result
135TNB	.21 UGL	310	70	43		31
13DNB	.458 UGL	0.905	4.16	LT	LT	23
246TNT	.426 UGL	3900	510		98	97
24DNT	.397 UGL		2900		1600	110
26DNT	.6 UGL	LT	LT	LT		230
Aluminum	112 UGL	3900	3700	14400	287	530
Arsenic	2.35 UGL	LT	LT	3.87	LT	
Barium	2.82 UGL	13.3	62.5	53.2	76.9	54
Beryllium	1.12 UGL	LT	LT	LT	LT	
Boron	230 UGL	LT	LT	LT	LT	
Cadmium	6.78 UGL	LT	LT	LT	11.9	
Calcium	105 UGL	20800	3520	76400	46300	73100
Chromium	16.8 UGL	LT	LT	LT	LT	
Cobalt	25 UGL	43.2	52.8	LT	115	LT
Copper	18.8 UGL	LT	LT	LT	LT	
Iron	77.5 UGL	3980	2020	11600	3120	490
Lead	4.47 UGL	LT	6.09	12.3	LT	
Magnesium	135 UGL	5390	1670	13900	15400	9780
Manganese	9.67 UGL	5870	1280	352	15000	1230
Mercury	.1 UGL	0.101	LT	0.13	0.116	0.22
Nickel	32.1 UGL	LT	LT	LT	97.6	
Potassium	1240 UGL	1510	2810	1670	LT	1710
Selenium	2.53 UGL	3.95	LT	LT	LT	
Silver	10 UGL	LT			LT	
Thallium	125 UGL	LT	LT	LT	LT	
Vanadium	27.6 UGL	LT	LT	LT	LT	
Zinc	18 UGL	26.6	28.6	79.5	64.4	36.6
Cyanide	5 UGL	35	LT	LT	18.2	5.34
Alkalinity	5000 UGL	37000	21000	200000	69000	220000
Alkalinity-Bicarbonate	5000 UGL	37000	21000	200000	69000	220000
Chloride	278 UGL	5180	6530	2460	3300	4030
Fluoride	153 UGL	2970	LT	297	637	888
Nitrate/Nitrit	10 UGL	4400	9700	1500	1600	9000
Sulfate	175 UGL	1500000	291	11000	140000	18000
COD	10000 UGL			LT		
TOC	1000 UGL			1300		
TDS	10000 UGL			300000		
TSS	4000 UGL			250000		

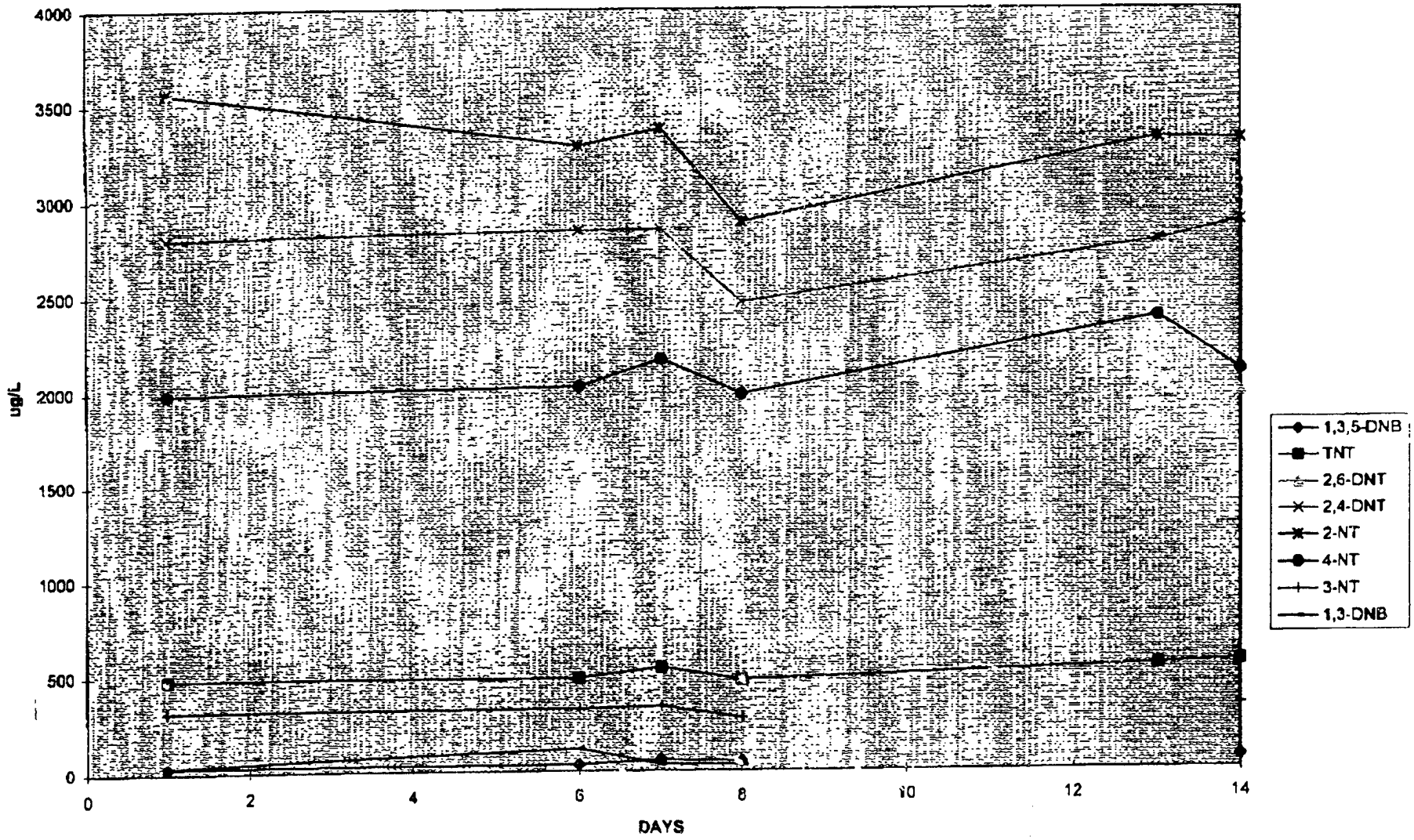
Table 4.15 RI/FS Data for Groundwater Monitoring Wells

	Well	85	86	88	91	110
Parameter	CRLs Units	Result	Result	Result	Result	Result
135TNB	.21 UGL	6.09		0.742	22	1100
13DNB	.458 UGL		12.6	LT		
246TNT	.426 UGL		5.89	3.98	15.1	570
24DNT	.397 UGL		71	2.22	87	4800
26DNT	.6 UGL	LT	26	LT	120	870
Aluminum	112 UGL	10800	265	LT	1260	641
Arsenic	2.35 UGL	2.79		LT		
Barium	2.82 UGL	90.7	24.8	17.2	12.2	11.8
Beryllium	1.12 UGL	LT		LT		
Boron	230 UGL	252		LT		
Cadmium	6.78 UGL	LT		LT		
Calcium	105 UGL	93800	38500	2610	NB	NB
Chromium	16.8 UGL	LT		LT		
Cobalt	25 UGL	LT	LT	LT	LT	25.6
Copper	18.8 UGL	LT		LT		
Iron	77.5 UGL	9670	239	201	1120	549
Lead	4.47 UGL	23.6		LT		
Magnesium	135 UGL	23800	7590	993	1620	633
Manganese	9.67 UGL	362	1510	344	687	2520
Mercury	.1 UGL	0.228	LT	LT	0.352	2.6
Nickel	32.1 UGL	LT		LT		
Potassium	1240 UGL	1890	1460	1580	LT	3770
Selenium	2.53 UGL	LT		LT		
Silver	10 UGL	LT		LT		
Thallium	125 UGL	LT		LT		
Vanadium	27.6 UGL	LT		LT		
Zinc	18 UGL	94.7	20.8	LT	LT	41.6
Cyanide	5 UGL	LT	LT	LT	LT	68.7
Alkalinity	5000 UGL	250000	190000	6100	91000	94000
Alkalinity-Bicarbonate	5000 UGL	250000	190000	6100	91000	94000
Chloride	278 UGL	3680	3130	1440	2400	5570
Fluoride	153 UGL	365	844	LT	1170	3250
Nitrate/Nitrit	10 UGL	4200	4100	1400	5300	53000
Sulfate	175 UGL	5150	25000	3170	64000	1100000
COD	10000 UGL					570000
TOC	1000 UGL					190000
TDS	10000 UGL					2700000
TSS	4000 UGL					4000

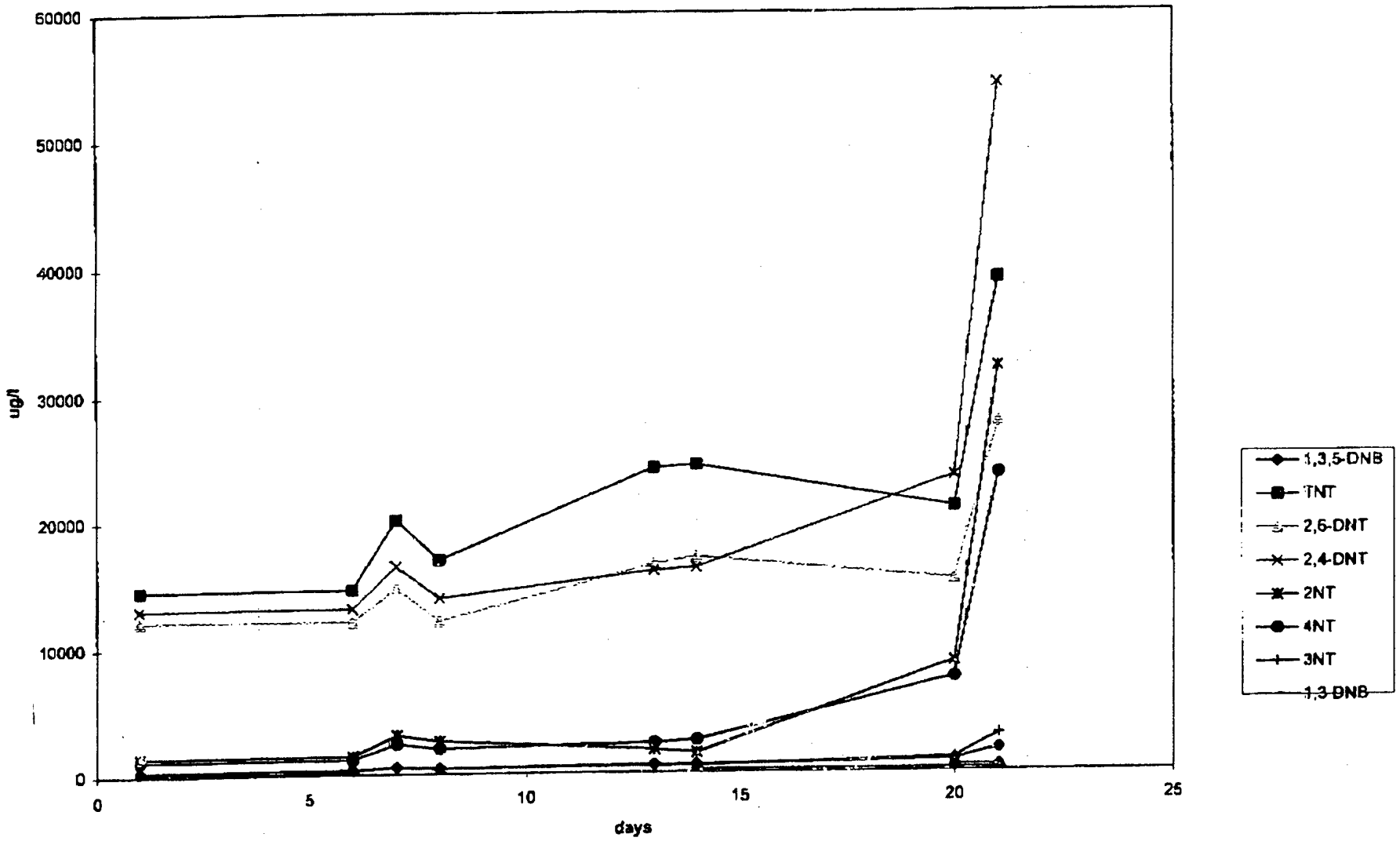
Plopt of Data from Well 40



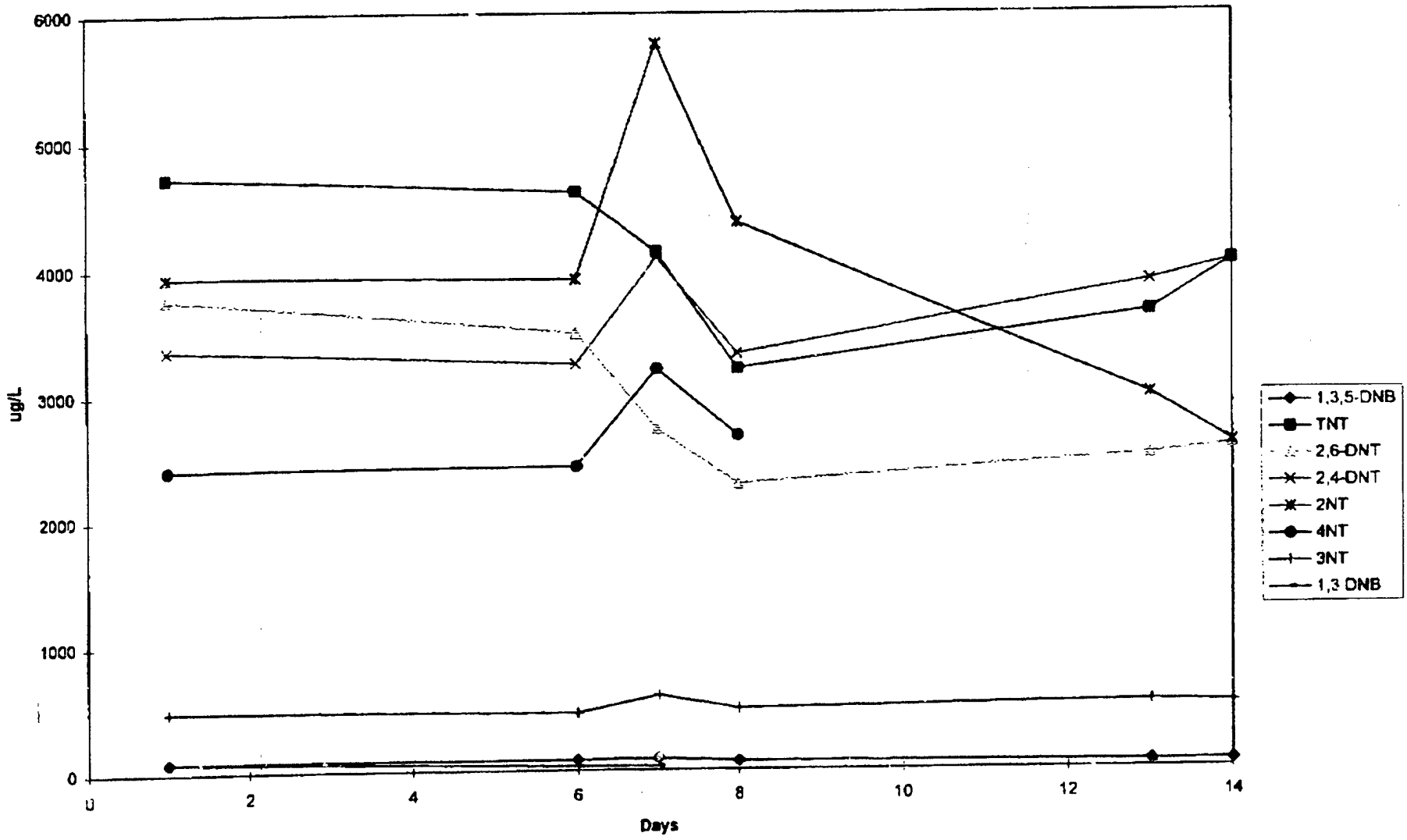
DATA FROM WELL 65



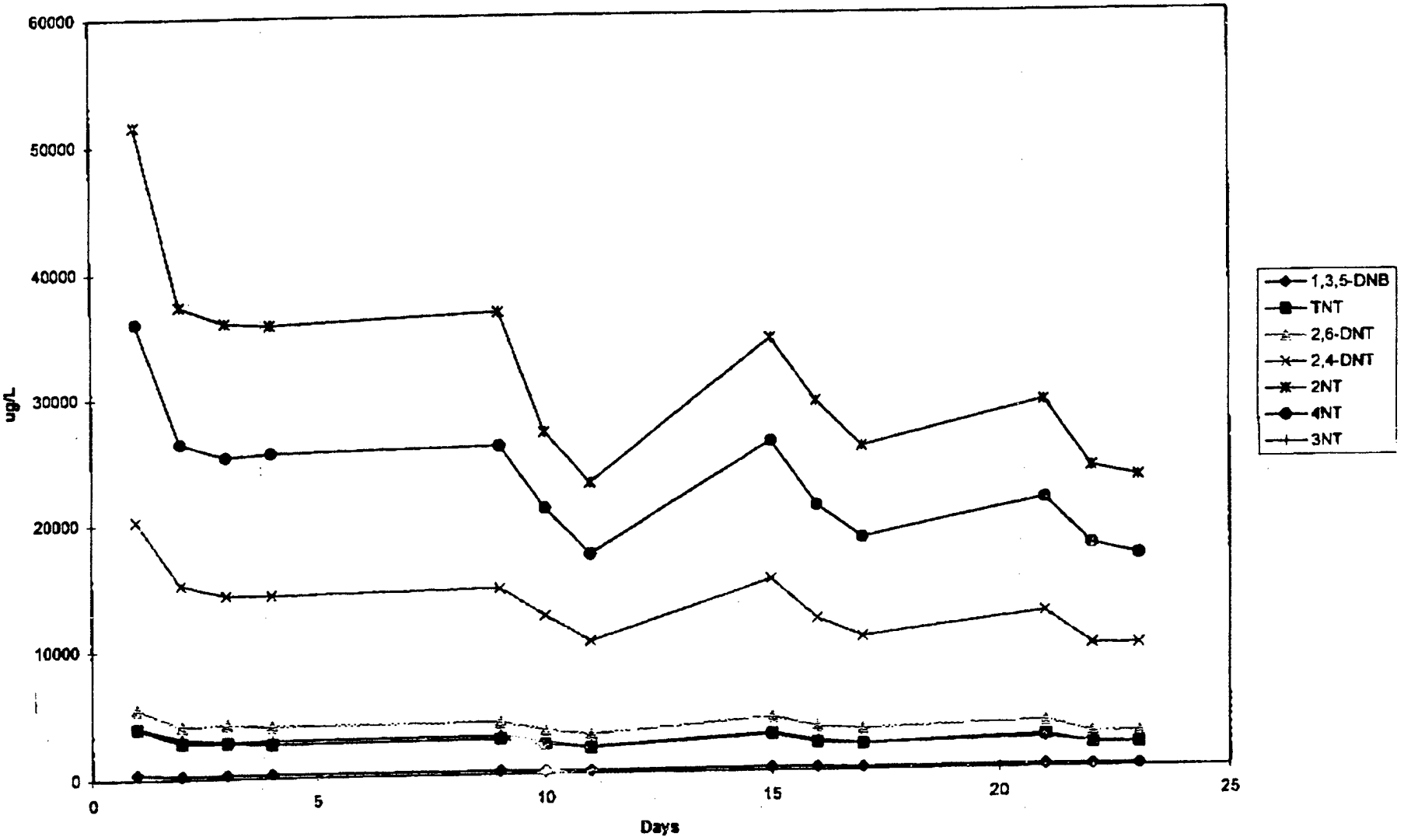
Plot of the Data from Well 66



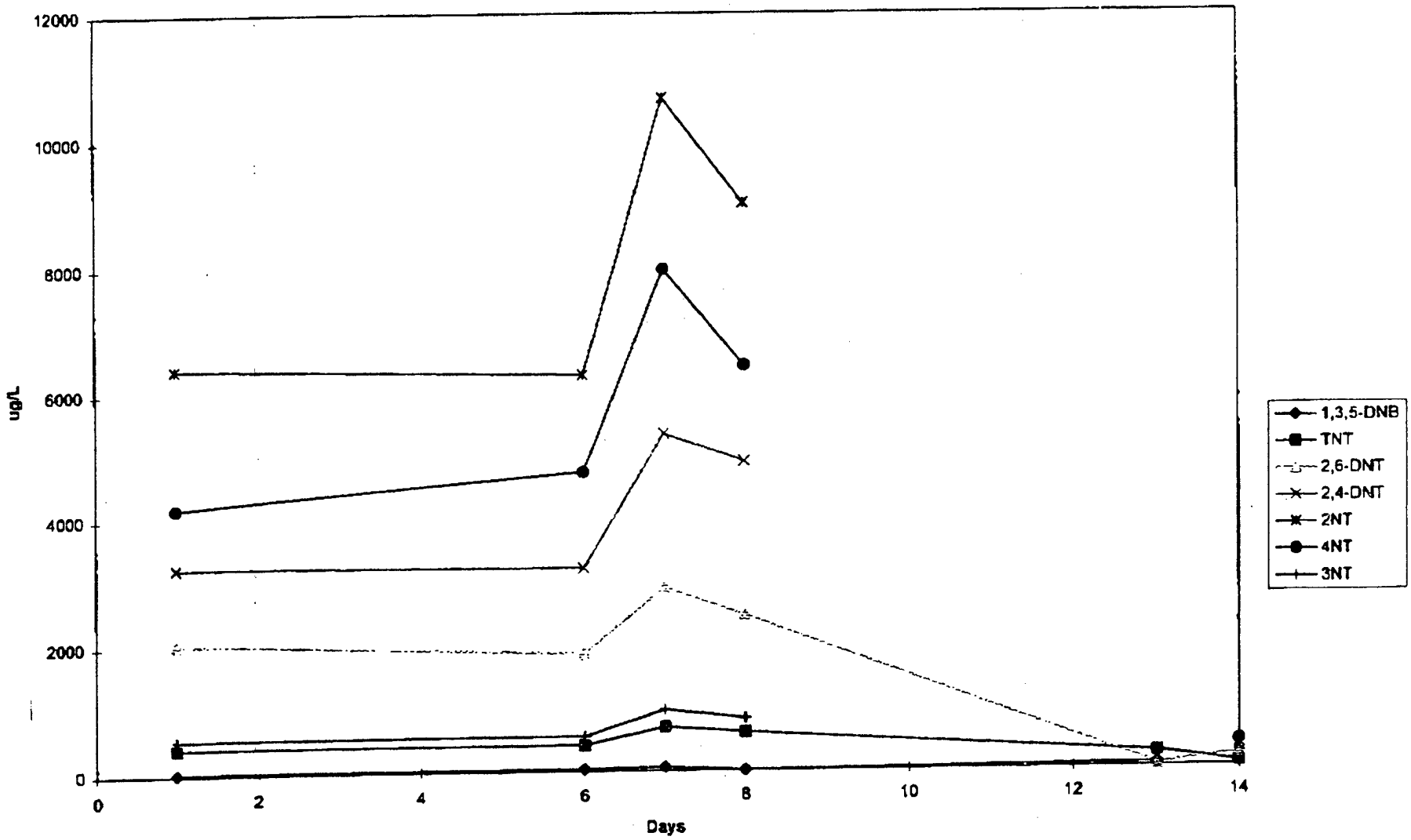
Plot of the Data from Well 69



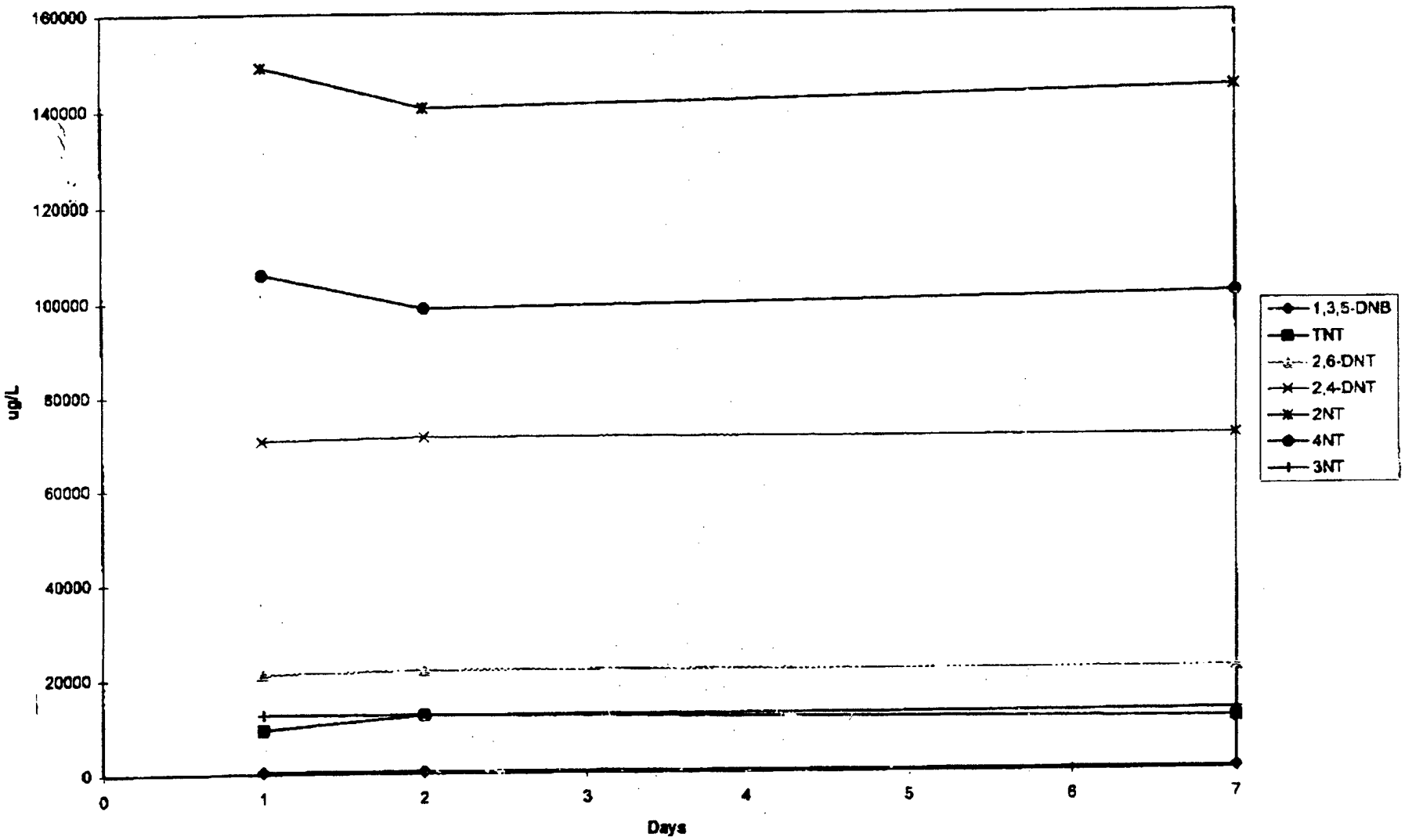
Plot of the explosive concentrations in Well 77



Plot of Data from Well 79



Plot of the Explosive Conc. in Well 92



Appendix B

Standard Operating Procedures and Data Sheets

Standard Operating Procedures for detection of the explosives TNT and RDX with the Continuous Flow Immunosensor. (Ver 1.0)

I. Supplies

Fast 2000
Coupons for Fast 2000
Functionalized membranes
Laptop portable computer
Buffers (see section II) supplies: sodium monophosphate, Tween 20, ethanol
Pipetman and tips (200µl and 1 mL)
Test tubes or vials
Disposable syringes
HPLC syringe (150 µl)
Acrodisc filters (0.45 µm)
Methanol
Deionized water

II. Flow Buffer Preparation:

10 mM Sodium Monophosphate
2.5% Ethanol
0.01% Tween 20

1. Dispense ~300 mLs of MilliQ double distilled water into a clean graduated cylinder.

2. Add 5.0mLs of stock 1.0M Sodium Monophosphate pH 7.2 to the graduated cylinder.

3. Add 12.5 mLs of 200 proof Ethanol to the graduated cylinder.

4. Add 500 uL of stock 10% Tween 20 (in MilliQ double-distilled water) to the graduated cylinder.

5. Bring the final volume of the graduated cylinder to 500 mLs using MilliQ double-distilled water.

6. Transfer the solution to a clean container with a cap until needed. If the flow buffer is not going to be used immediately, it should be stored at 4^0C.

III. Explosive Standard Preparation

1000 ng/mL	50 ng/mL
500 ng/mL	25 ng/mL
250 ng/mL	10 ng/mL
100 ng/mL	5 ng/mL

1. Take a stock concentration of TNT/RDX (1000 ug/mL in acetonitrile) commercially available from a reputable analytical reference materials supplier.

2. Add 20 uL of the stock to a clean 13X75 mm test tube.

3. Air dry the 20 uL containing the standard.

4. Add 2.0 mLs of flow buffer to the test tube containing the standard for a final concentration of 10 ug/mL.

5. Make the appropriate dilutions in 13X75 mm test tubes using flow buffer and seal standards using parafilm. Store the standards away from light until needed.

IV. Sample Preparation

Aqueous Sample Preparation

1. Using a Concentrated Flow Buffer comprised of the following:

   0.63M Sodium Monophosphate
   0.5% Tween 20

2. 37.5 uL of Ethanol (200 proof) is added to 30 uL of the Concentrated Flow Buffer and dispensed into a 13 X 75 mm test tube.

3. 1432.5 uL of aqueous sample is added to the above mixture.

4. The sample is mixed thoroughly and then covered and stored out of direct light until needed for analysis.

5. If the samples apprear to have a yellow color it is recommended that they be scanned for an excitation / emission wavelength to determine the relative background fluorescence which will interfere with the assay.

V. Fast 2000 Instrument Setup

A. Filling the Buffer Bag with Flow Buffer

1. Remove the Buffer Bag (located in the receptacle with the Plexiglas cover) by disconnecting the tubing. Care should be taken to keep the keep the bag upright to prevent spillage.

2. Fill a 60 mL syringe with flow buffer.

3. Attach a 0.4 um filter using the lure lock connection to the syringe.

4. Place tubing to connect the filter-end of the syringe to the buffer bag.

5. Slowly depress the syringe taking care not to rupture the filter.

6. Repeat the procedure two times for a total volume of 180 mLs.

7. Remove the syringe and tubing from the bag and reattach system tubing to the buffer bag and place the bag in the buffer reservoir.

B. Coupon Preparation

1. Place the coupon such that the membrane cover is facing up, two small notches should be visible on either side of the cover. The rubber septum should also be facing up.

2. Using a small screwdriver, pry the membrane cover off.

3. Remove the old membrane and filter and discard.

4. Place a single charged membrane into the PLUG of the coupon followed by a filter and insert into the coupon. The cover should be flush against the surface.

C. Connecting the FAST 2000 to the Bulkhead and Computer

1. Plug the DAQ card into the PCMCIA (type II) slot of the laptop computer (486 100MHz / 24 Meg RAM, or better). User should have familiarity with Windows Operating Systems.

2. Plug the laptop into a power source if available.

3. Remove the plugs from the ports located on the side of the FAST 2000.

4. Attach the color-coded tubing from the Bulkhead to the FAST 2000 (blue to blue, green to green, etc.)

5. Turn on the computer and <Double-Click> the FAST 2000 icon to begin the software.
Note: *the first screen should default to running in the "REAL MODE" if all of the computer connections are made correctly.*

6. Task Manager will appear on the upper left hand portion of the screen.

7. Prompt will highlight the "Flush Buffer to Establish Baseline". <Click NEXT>.

8. When prompted, insert the coupon into the carriage (under the lid) and close the retaining bar to position the coupon securely. <Click> Box next to the message to confirm.

9. When prompted, open all valves at the bulkhead. <Click> Box next to the message to confirm. The coupon priming function will be initiated and followed by the Buffer Flush.

10. Check the blue and yellow tubing to make sure buffer is flowing through the system.

11. <Click> the <NEXT> box when the baseline is acceptable. (In order to view the baseline, it will be necessary to Rescale the graph with the button located on the lower right-hand side of the screen). The original Task Manager Screen will be displayed.

VI. Running an Assay and Analyzing the Data

1. Select the Inject sample and run assay and <Click> the <Next> button.

2. Using a HPLC syringe, measure 150uL of the sample that you want to inject making sure to remove any air bubbles from the syringe. Insert the tip into the septum located on the top surface of the coupon and depress the syringe to load the sample.

3. After injecting the sample, rinse the syringe with two volumes of methanol followed by two volumes of MilliQ water to decontaminate.

4. <Click> the box to confirm the injection has been completed.

5. When the baseline is acceptable, (rescaling the graph may be necessary) <Click> the Begin Assay box in the Task Manager. The assay will run for approximately 2 minutes.

6. When the assay is complete, a File Parameters dialogue box will appear on the top of the screen which will prompt the user to save the data acquired during the assay.

7. If the user chooses to save the data, the File Parameters information should be entered followed by <Clicking> the <Save Data> box in the Task Manager box.

8. <Click> the <Next> box to continue. Note: Multiple assays can be performed in succession and stored to be analyzed at a later time.

9. To analyze any saved data from previous assays, <Click> the Analyze data option in the Task Manager followed by the <Next> button.

10. Select the Data File option to retrieve the desired file from the menu. <Double Click> the file that is to be analyzed.

11. Using the pointer, delineate the portion under the peak that represents the displaced antigen by <clicking> on the start and end points on the curve. The area will be displayed in the Integral box in the Task Manager.

12. Save the analyzed data to a file by <Clicking> <yes> in the dialogue box. Note: if the user has used the smoothing function during the analysis of the data, the original data can not be retrieved.

VII. System Shutdown

1. <Click> the System shutdown option from the Task Manager followed by the <Next> button.

2. Close all bulkhead valves and confirm by <Clicking> the box in the Task Manager.

3. Eject the coupon by raising the lever on the FAST 2000 and sliding the coupon out. Remove the membrane and filter and discard. Confirm the action in the Task Manager.

4. Attach the DI water bottle to the bulkhead by attaching the color-coded tubing to the appropriate connections on the instrument. The waste line will remain in place to divert flow of the wash to the waste container.

5. Reinsert the empty coupon into the FAST 2000 and confirm action by <Clicking> the box in the Task Manager. Make sure that the waste valve is in the open position. The rinse of the system will begin.

6. Close waste valves to prevent backflow of waste into the instrument. Confirm the action in the Task Manager.

7. Disconnect the tubes from the FAST 2000 and cap all bulkheads. Confirm the action in the Task Manager.

8. Empty and clean all fluid containers.

9. Remove the coupon and shutdown the computer.

Rev. 7/97

NRL FAST 2000 DATA SHEET

	Date	Time	No. of Samples	Matrix Type	Remarks / Condition of Samples
Received from:					
Received by :					

Sample Identification	Sample Preparation/Initials	Date	FAST2000 Filename / Initials	Comments / Concentration